Future Direction of Genome Medicine in Hematologic Malignancies

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Although the healthcare system for cancer precision medicine has been established in Japan for the past few years, it mainly focuses on solid cancers. In hematologic malignancies, such as leukemia and lymphoma, driver genes targeted by somatic alterations are different from those in solid cancers. Clinical sequencing has been performed mainly for identifying therapeutic targets in solid cancers. Besides this purpose, it is useful in diagnosis and prognostic prediction for hematologic malignancies. Therefore, different strategy and next-generation sequencing (NGS) panel are needed for precision medicine in hematologic malignancies. Here I summarize the clinical relevance of somatic alterations and the underlying scientific basis in hematologic malignancies.

Key Words : Precision Medicine, Hematologic Malignancy, Next-Generation Sequencing (NGS) Panel, Adult T-cell Leukemia/Lymphoma, Whole-Genome Sequencing

Introduction

In Japan, recent years have seen a rapid advancement in the establishment of a healthcare system for cancer precision medicine, with gene panel tests introduced into clinical settings in the fiscal year 2019. Hematologic malignancies and solid tumors differ in therapeutic target genes; for the former, gene panel testing is important for making diagnosis and prognostic prediction, in addition to treatment selection, which is the main purpose of this testing for the latter. Currently, gene panel testing is employed mainly for solid tumors, and hematologic malignancies are lagging behind in this regard. In particular, the non-availability of insured gene panel tests for hematologic malignancies is a major problem in our country. Here I summarize the clinical relevance of somatic alterations and the underlying scientific basis in hematologic malignancies.

Genomic abnormalities in malignancies

All malignancies, including hematopoietic ones, arise through the acquisition of a somatic cell abnormality that causes a functional change in genes related to cancer development and progression. Large-scale comprehensive genome sequencing has been done lately in the projects by The Cancer Genome Atlas of the United States (TGGA), the International Cancer Genome Consortium (ICGC) comprising cancer research institutes worldwide, and others[1,2]. In Japan, we have mainly focused our genetic analysis on adult T-cell leukemia/lymphoma (ATL), a common malignancy among Japanese, and have identified various new genomic abnormalities[3–5]. These efforts together have led to the accumulation of genomic analysis data from several hundreds of thousands of cases regarding malignancies of over 100 different histologic types, and the collection of

such data is continuously expanding. Using these data, the whole picture of genomic abnormalities (e.g., genetic variants, copy number variants, and chromosomal rearrangements) is being uncovered, and the biological process that leads to oncogenesis is being unraveled. Additionally, several potential therapeutic targets and biomarkers that affect treatment responses and outcomes have been identified.

Cancer genomic medicine: precision medicine

"Cancer genomic medicine" refers to medicine that provides "personalized care" suited to individual patients' constitutions and disease states, capitalizing on their "genomic information" identified by next-generation sequencing (NGS) and other measures. To be specific, results of quality- and reliability-assured genomic testing and other various health information are used as a basis to establish a diagnosis, to select the most promising preventive and treatment strategies, and to estimate the disease onset for each individual. Taking an example of the epidermal growth factor receptor (EGFR) inhibitor gefitinib, public health insurance coverage was first approved in the treatment of inoperable nonsmall cell lung cancers, but the response rate remained 20%–30%. This drug was later found to be effective only in treating nonsmall cell lung cancers with *EGFR* gene mutation, and the indication was changed accordingly. The response rate then increased up to approximately 70%–80%. This episode indicates that selection and stratification of probable responders among patients based on their genomic information can lead to the avoidance of medical therapy to poor responders and therefore higher drug effectiveness. Examination for single gene abnormalities had been possible using companion diagnostics; recently, the advent of NGS has enabled simultaneous assessment of numerous cancer-related genes.

Genomic medicine for hematologic malignancies

Regarding genomic testing for solid cancers, the "Clinical practice guidance for NGS in cancer diagnosis and treatment (Edition 1.0)" was jointly released in 2017 by the Japanese Society of Medical Oncology, the Japanese Society of Clinical Oncology, and the Japanese Cancer Association. For hematologic cancers, the Japanese Society of Hematology (JSH) issued separate guidelines in 2018, the "Guidelines for genomic testing for tumors of hematopoietic and lymphoid tissues", based on their standpoint on genomic testing for hematologic malignancies, which differed from that reflected in the above solid tumor guidelines. The JSH guidelines provide not only clinically significant gene mutations in hematologic cancers selected on the basis of the latest published evidence and information that constitute the basis of gene panel testing-based genomic medicine, but also detailed descriptions of its clinical utility. Gene panel testing in hematologic malignancies is especially distinct from that in solid tumors in its usefulness in diagnosing and predicting prognosis, in addition to selecting treatment, which is the main purpose of this testing in solid tumors (**Fig 1**). Specific potential clinical

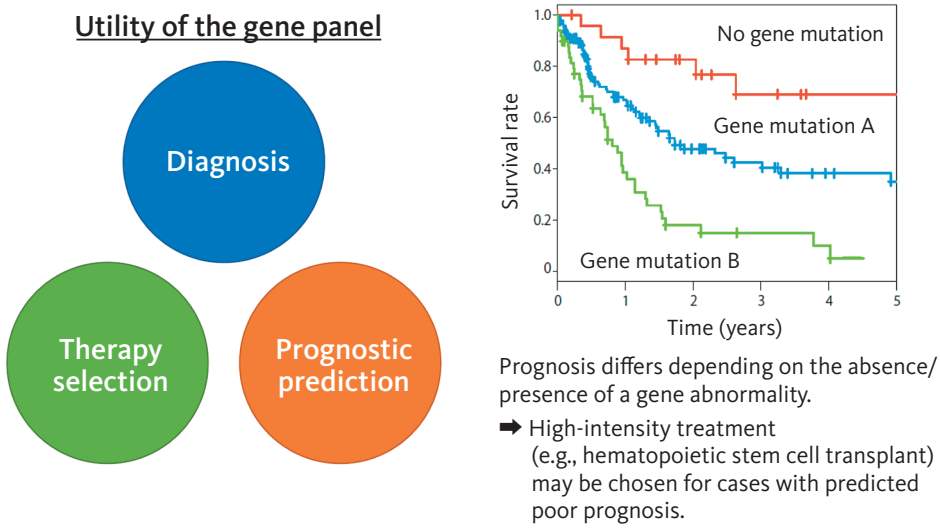

Fig 1. Utility of the gene panel in hematologic malignancies

utilities of genomic medicine for hematologic malignancies are described below. These utilities have been validated in several prospective studies on the clinical use of gene panel testing[6–8].

Treatment selection

The treatment of many cancers, including hematologic ones, is provided according to the classification of the disease based on its origin and pathological tissue. Response to treatment widely varies among individuals. Recently, laboratory and clinical evidence is accumulating that individual patients' genomic alterations can be therapeutic targets and that the treatment response is predictable by the type and intensity of the alterations[9,10]. What counts here is that such genomic alterations may be used as molecular markers for identifying potential responders to specific treatments. A hallmark example of this is the use of imatinib, a tyrosine kinase inhibitor, toward the *BCR-ABL* fusion gene in chronic myeloid leukemia[11]. Other examples include the use of FLT3 inhibitors toward *FLT3* mutation-positive acute myeloid leukemia (AML) and IDH2 inhibitors toward *IDH2* mutation-positive AML. The former treatment is covered by the national health insurance in Japan while the latter is a United States Federal Drug Agency (FDA)-approved therapy. In molecular targeted therapies for genetic abnormalities, acquisition of treatment resistance via a secondary genomic mutation in a relevant pathway has also been reported. A typical example of this is secondary mutations within the *ABL* gene, such as T315I mutation, that can occur during imatinib therapy for *BCR-ABL*[11]. In the future, NGS assay is expected to promote the elucidation of secondary genomic abnormalities like these and, in line with this, the development of next-generation molecular targeted therapies to overcome treatment resistance associated with such abnormalities.

Diagnosis and prognostic prediction

Large-scale sequencing of various forms of hematologic malignancy has contributed to the discovery of genomic alterations that can be new therapeutic targets and their application in clinical treatment and advances in genomic alteration-based molecular classification of the disease and integration of the further refined classification into diagnostic and prognostic decisions[12]. The 2017 revised edition of *WHO Classification of Tumours and Haematopoietic and Lymphoid Tissues* includes more than 300 genomic alterations. Especially in AML, the subset with recurrent genomic alterations (e.g., fusion genes, such as *RUNX1-RUNX1T1* and *CBFB-MYH11* and mutations in genes, such as *NPM1* and *CEBPA*) is classified as a separate entity[13]. Additionally, for myeloproliferative neoplasms, myeloid/lymphoid neoplasms with eosinophilia, myeloid neoplasms with germline variants, acute lymphoid leukemia, and high-grade B-cell neoplasms, among others, certain subtypes are defined by specific genetic alterations. Given these, comprehensive diagnosis incorporating genetic alterations is becoming essential for hematologic malignancies. Also, genomic alterations useful in the diagnosis but do not define disease subtypes are presented, e.g., *BRAF* and *MAP2K1* mutations in hairy cell leukemia and the *MYD88* mutation (L265P) in lymphoplasmacytic lymphoma[13]. These genomic alterations have been reported to be also useful in predicting prognosis. For instance, in AML, an *NPM1* mutation with no *FLT3* mutation and a biallelic *CEBPA* mutation are indicators of a favorable treatment outcome, whereas an *FLT3* mutation is associated with a poor outcome. As for malignant lymphomas, high-grade B-cell lymphoma is diagnosed in the presence of *MYC* and *BCL2* or *BCL6* translocations, and such cases are known to be associated with a poor prognosis. In the future, it is expected in clinical practice that genomic alterations would serve as a predictor of a prognosis and that treatment is to be selected on the basis of the estimated outcome.

Whole-genome sequencing (WGS) in ATL

As stated previously, numerous genetic analysis studies conducted to date have shed light on the whole picture of genomic alterations in various types of cancer, which now serves as the scientific basis of the current genomic medicine. Especially, many WGS studies conducted recently have identified driver gene abnormalities, including noncoding region alterations and structural variations (SVs), as bases of cancer development and progression. Under the circumstances, we recently executed WGS in ATL[5], which is a rare but fatal peripheral T-cell malignancy. This disease is caused by human T-cell leukemia virus type 1 (HTLV-1), which infects approximately 10 million individuals around the world. In Japan, HTLV-1 is prevalent in southwestern areas such as Kyushu and Okinawa, making ATL a common condition among the Japanese population. We performed WGS on 150 patients with ATL and obtained a comprehensive picture of genomic alterations in them. Particularly important discoveries in this investigation are described below in detail.

Discovery of frequent inactivations of the CIC–Ataxin 1 (ATXN1) complex

Using driver mutation discovery algorithms of MutSig2CV, DriverPower, and dNdScv, we identified 47 significantly mutated genes, including 10 novel ones. Surprisingly, alterations were discovered at high frequencies (33%, mutations and SVs combined) in the *CIC* gene, which had been previously unreported (**Fig 2**). *CIC* is a transcriptional repressor with the high mobility group (HMG)-box domain. There are two known isoforms of *CIC*, long (CIC-L) and short (CIC-S) forms. Almost all (95%) mutations were in the exon specific to the recently discovered isoform *CIC-L* (exon 2). This exon is in a region that has not been previously covered by whole-exome sequencing studies. More than half of the *CIC* mutations in ATL were loss-of-function mutations. While *CIC* mutations have been reported in low-grade glioma (LGG) and certain forms of adenocarcinoma, *CIC* mutations discovered in ATL differed in the type and distribution compared to other cancers. In LGG, the mutations were located mainly in exons common to both the long and short isoforms. However, in gastrointestinal adenocarcinomas (stomach and colorectal cancers), the mutations were distributed over the entire *CIC* gene, including the *CIC-L*-specific exon. By taking *CIC-L* mutations into consideration, the mutation frequency

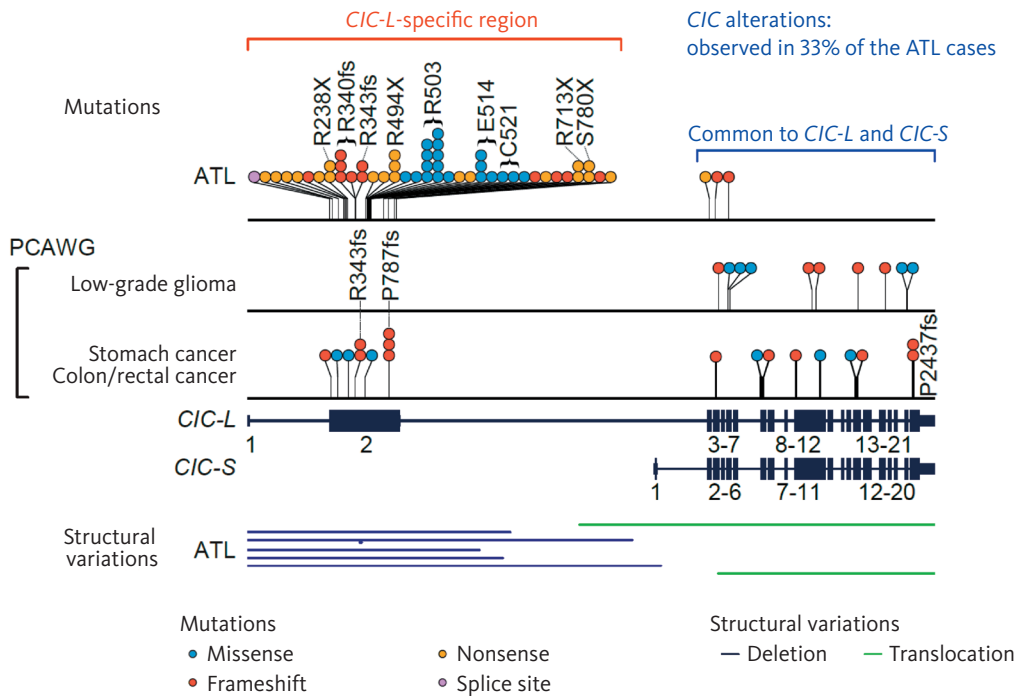

Fig 2. Distribution of *CIC* mutations in ATL and other cancers

Adopted from Kogure Y, Kameda T, Koya J, et al. Whole-genome landscape of adult T-cell leukemia/lymphoma. Blood. 2021. with partial modifications

increased than previously reported. In ATL, when the SVs mainly disrupting the *CIC-L* structure were included, *CIC* mutations were present in 49 (33%) cases, almost all (78%) of which were biallelic.

CIC forms a transcriptional repressor complex with ATXN1 and the complex's gain-of-function via polyglutamine-expanded ATXN1 causes spinocerebellar ataxia type 1 (SCA1) neurodegeneration. Interestingly, our assessment of driver mutations' coexisting/mutually exclusive relationships found mutual exclusivity between *CIC* and *ATXN1* alterations to be the most pronounced. This seems to reflect a functional relationship between CIC and ATXN1. In addition to the previously reported loss-of-function mutations and deletions of ATXN1, SVs of ATXN1 were found in 20% of the cases studied in our WGS. Furthermore, 53% of the cases had mutations in either *CIC* or *ATXN1*, suggesting that the CIC–ATXN1 complex has an important part to play in the onset of ATL.

To clarify the functional impact of CIC-L dysfunction, we produced conditional knockout mice (*Cic*-S and *Cic-L* cKO mice) of *Cic* short and long isoforms, respectively, and crossed them with Cd4-Cre transgenic mice. Surprisingly, $CD4^+CD25^+CD127^-Foxp3^+$ T-cell counts did not change in the spleen of the *Cic-S* cKO animals, as opposed to the *Cic-L* cKO mice, which showed nearly two-fold increases in the T cells. This result indicated that *CIC-L* deletion induced T-cell activation or regulatory T-cell increase. The above findings suggest that CIC-L plays a selective and important role in regulating the physiological state of T cells.

Identification of SVs truncating *REL* C-terminally in ATL and diffuse large B-cell lymphoma (DLBCL)

To distinguish between SVs in the sites of genomic instability and gain-of-function SVs, we analyzed SV breakpoint frequency per intron and found a highly significant accumulation of breakpoints in intron 7 of the *REL* gene. *REL* encodes the c-Rel protein, a Rel/NF-κB family transcription factor, and is deeply involved in the functions of T and B cells. Although *REL* copy number amplification has been reported in multiple forms of B-cell lymphoma, such as germinal center B-cell (GCB) DLBCL, the SVs of *REL* found in this study have not been fully investigated. In the case of ATL, 19 (13%) cases harbored SVs of *REL*, mainly in intron 7 and also in exon 10 (**Fig 3**). While various types of SVs were observed, e.g., deletions, inversions, and translocations, all cases had allylic variants of *REL*, with loss of the normal 3' region of several exons (exons 8–10) and its replacement with ectopic sequences. These SVs not only increased the *REL* expression but also produced abnormal transcripts of the gene. Specifically, in almost all cases, exons 1–7 and part of intron 7 were transcribed and fused into intronic or intergenic sequences containing a polyadenylation signal.

Since generally *REL* copy number amplifications are frequently observed in GCB-DLBCL, we searched the National Cancer Institute Center for Cancer Research's RNA sequencing data of 481 sample DLBCL cases for abnormal *REL* transcripts similar to those in ATL. In this cohort, no abnormal *REL* transcripts were found in cases of activated B-cell-type DLBCL, whereas 16

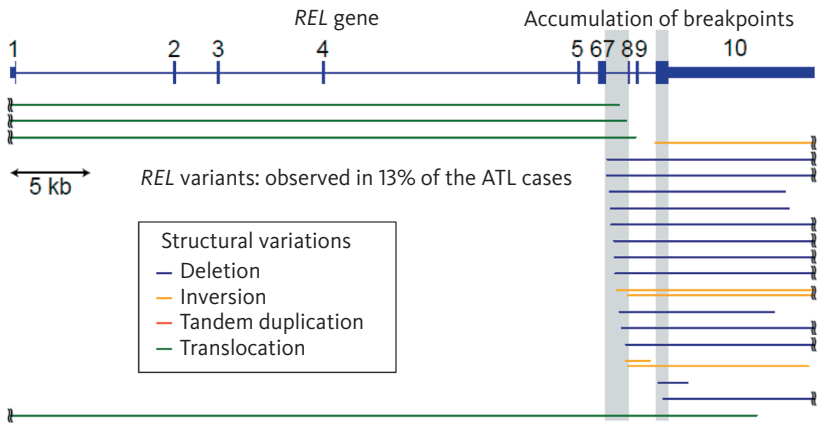

Fig 3. *REL* SVs due to truncations in ATL

Adopted from Kogure Y, Kameda T, Koya J, et al. Whole-genome landscape of adult T-cell leukemia/lymphoma. Blood. 2021. with partial modifications

(12%) cases of the GCB type and 6 (6%) cases of the unclassifiable type expressed such abnormal transcripts. Moreover, irrespective of the copy number state, *REL* SVs were related to increased *REL* expression levels. In ATL and DLBCL cases together, the intact *REL* coding regions were conserved in only five cases among those with *REL* SVs, while in almost all of the remaining cases, the coding sequence was interrupted at the end of exon 7 or within exon 10, generating a truncated protein. All proteins predicted from *REL* abnormal transcripts lacked the transactivation domain but retained almost the entire Rel homology domain.

Recurrent truncations of *REL* in specific regions suggest the resulting protein's gain-of-function. Therefore, we evaluated the C-terminally truncated c-Rel protein for its biological function. In the luciferase reporter assay, the truncated c-Rel protein, as opposed to the wildtype c-Rel, did not promote the transcription of NF-κB, showing a dominant-negative effect against the wildtype. However, where RelA, another NF-κB subunit, was coexpressed, not the wildtype but only the truncated c-Rel promoted the activation of NF-κB more than did RelA alone. These results indicate the presence of a synergistic effect between RelA and the truncated c-Rel. Moreover, RNA sequencing in samples from patients with DLBCL demonstrated an increased NF-κB gene signature in patients with *REL* SVs. In the DLBCL cell line harboring *REL* SVs, REL knockout via CRISPR inhibited cellular proliferation. The above findings suggest that recurrent *REL* SVs in ATL and DLBCL not only increase *REL* mRNA expression but also generate c-Rel protein with gain-of-function and serve as a cancer driver for ATL and DLBCL.

Whole landscape of ATL driver abnormalities

Analyses of coding mutations, copy number variations, SVs, and noncoding mutations together revealed significant alterations in 56 genes in ATL, which included significant coding mutations, copy number variations, SVs, and noncoding mutations in 47, 13, 13, and 6 genes, respectively. When all types of abnormalities were considered, more than 10% of cases harbored alterations in 32 genes. The median number of driver abnormalities was nine per case, with at least one abnormality in 149 (≥ 99%) cases. Of all driver abnormalities, coding mutations, copy number variations, SVs, and noncoding mutations accounted for 65%, 11%, 27%, and 4%, respectively. Abnormalities in four driver genes, including *ATXN1* and *REL*, were mostly (≥ 85%) SVs. When compared between disease subtypes, the total number of mutations and the numbers of SVs, copy number variations, and driver abnormalities were higher in the acute and lymphoma subtypes than in the chronic and smoldering subtypes. However, except for *STAT3*, which were predominantly mutated in the chronic and smoldering subtypes, there were no subtype-specific driver genes. Our WGS analysis provided profiles of drivers distinct from the ones reported in earlier studies, including frequent occurrences of driver abnormalities in more patients than previously understood.

We also identified two molecular groups in 56 driver abnormalities using consensus clustering with non-negative matrix factorization. Group 1 was associated with a small number of mutations, SVs, and driver and frequent abnormalities in proximal T-cell receptor (TCR) signaling molecules (e.g., *PLCG1*, *VAV1*, *CD28*, and *RHOA*) and *STAT3*. Group 2 carried abnormalities predominant in certain distal TCR/NF-κB signaling pathway constituents (*PRKCB* and *IRF4*), immune-related molecules (*HLA-A*, *HLA-B*, and *CD58*), and epigenetic regulatory factors (*EP300* and *TET2*). Almost all cases of lymphoma were classified into group 2, whereas group 1 mainly comprised cases of leukemic phase ATL. Furthermore, compared with group 1, group 2 was shown to have a poor prognosis regardless of the clinical subtype. These results indicate the biological and clinical importance of the WGS-based molecular classification of ATL.

Conclusions

Prospective research on the clinical utility of gene panel testing in hematologic malignancies conducted in Japan is one step forward toward the development and national health insurance coverage of such testing[6]. However, as of September 2021, no insured gene panels are available for hematologic malignancy analysis in this country. In the United States, a comprehensive genomic profiling test FoundationOne® Heme has been developed by Foundation Medicine, Inc. but the test is not FDA approved. To ensure successful clinical application of genomic medicine for hematologic malignancies in Japan, it is essential to develop gene panel testing that targets these conditions. Additionally, various challenges remain to be cleared, including fostering professionals as members of an expert panel that provides medical interpretations of panel test results

and establishing a follow-up system for familial myeloid malignancies, which have been recently found to occur at a relatively high frequency. Also, to further refine genomic medicine, it will be necessary, as suggested by WGS in ATL, to unveil genetic alterations using advanced genetic analysis technologies and, capitalizing on outcomes thereof, to elucidate the molecular pathology of diseases. Efforts shall be required to establish scientific foundations supported by basic research.

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