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Clinical Application of Chromosomal Microarray for Hematologic Malignancies

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Chromosomal microarray (CMA) can detect genome-wide small copy number abnormalities (CNAs) and copy-neutral loss of heterozygosity (CN-LOH) better than conventional karyotyping and fluorescence in situ hybridization (FISH) for hematologic malignancies. Apart from the limitations in detecting balanced chromosomal rearrangements and low-level malignant clones, CMA has clinical utility in detecting significant recurrent and novel variants with diagnostic, prognostic, and therapeutic evidence. It can successfully complement conventional cytogenetic tests for several hematological malignancies, including acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). An increase in CMA testing for hematologic malignancies is expected to identify novel markers of clinical significance.

Key words: Chromosomal microarray, Hematologic malignancy, Copy-number abnormalities, Copy-neutral loss of heterozygosity

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INTRODUCTION

Conventional karyotyping is the primary test used to detect chromosomal aberrations in hematological malignancies. This is useful for detecting numerical aberrations and balanced rearrangements [1]. However, karyotyping has limitations, such as low resolution, lack of objective parameters to define G-banding patterns, and dependence on cell culture efficiency. Fluorescence in situ hybridization (FISH) can overcome the limitations of karyotyping. However, FISH can be used only for specific chromosomal regions [2].

Chromosomal microarray (CMA) is widely used to detect small copy number variants (CNVs) and is primarily recommended for application in germline disorders such as neurodevelopmental disorders and congenital malformations [3]. CMA can detect copy-neutral loss of heterozygosity (CN-LOH) using single nucleotide polymorphism (SNP) markers, and is applicable for diagnosing imprinting disorders [4]. CMA is also applicable to the genetic diagnosis of somatic disorders such as hematologic malignancies and lymphomas [5,6]. In this article, we describe the clinical utility of CMA in the genetic investigation of hematologic malignancies.

BENEFITS AND LIMITATIONS OF CMA FOR HEMATOLOGIC MALIGNANCIES

Recent CMA platforms consist of CNV and SNP markers. The CNV compares the scanned data of the sample with the control data, which were obtained using hundreds of control individuals [7]. Therefore, the application of a control sam-

ple is not required, which is required for conventional array comparative genomic hybridization (aCGH) [8]. SNP markers are complementary to CNV markers and help detect CNVs more accurately using B-allele frequencies [9]. A combination of CNV and SNP markers can be used to distinguish between heterozygous deletions and CN-LOH [10].

The application of CMA to hematological malignancies does not require cell culture processing, which is crucial for karyotyping and FISH. Therefore, CMA can avoid cell culture bias and may have a shorter turnaround time than karyotyping [10]. Most CMAs have a higher resolution than karyotyping and are much more sensitive for detecting small copy number abnormalities (CNAs) with sizes < 5–10 Mb [11]. It can also discern complex chromosomal abnormalities such as amplification, chromothripsis, intrachromosomal complexity, and genomic complexity [12]. The distinction between the doubling of hypodiploid clones of acute lymphoblastic leukemia (ALL) and non-hypodiploid ALL can be achieved by CMAs [10].

However, CMA has certain limitations. Generally, balanced rearrangements cannot be detected, such as balanced translocations or inversions. The CMA results depend on the proportion of malignant cells in the sample, and has limitations in detecting minimal residual diseases (Table 1). This is because CMA cannot detect low levels of mosaicism or chimerism with a percentage < 20% [13]. CMA cannot distinguish between individual clones, such as stemlines and sidelines either [14]. CMA is not recommended for all types of hematologic malignancies [15]. The interpretation of CMA results can be difficult for hematologic malignancies compared to germline disorders because the public database is limited to somatic CNAs [12].

INDICATIONS OF HEMATOLOGIC MALIGNANCIES FOR CMA

All hematological malignancies were not indicated in the CMA analysis. Generally, the diagnostic and prognostic benefits of CMA are limited to chronic myelogenous leukemia (CML) and myeloproliferative neoplasms (MPN) [15]. However, CMA can sensitively detect recurrent or novel findings in acute myeloid leukemia (AML), myelodysplastic syndrome

Table 1. Comparison of advantages and disadvantages in cytogenetic tests for hematologic malignancies

Method	Advantages	Disadvantages
Karyotyping	- Direct observation of all chromosomal abnormalities - Can detect balanced translocations	- Low resolution - Requires cell culture - Cannot detect small CNAs
FISH	- High sensitivity for specific chromosomal abnormalities	- Limited to predefined regions - Requires pre-designed probes
CMA	- High-resolution detection of CNAs - No need for cell culture - Can detect CN-LOH	- Cannot detect balanced translocations - Depends on the proportion of malignant cells - Limited ability to detect low-level mosaicism

FISH, fluorescence in situ hybridization; CMA, chromosomal microarray; CNA, copy number abnormality; CN-LOH, copy neutral loss of heterozygosity.

Table 2. Indications and suggestive findings of hematologic malignancies for chromosomal microarray

Indication	Suggestive findings
AML	-5/5q del, -7, KMT2A partial tandem dup, 13q CN-LOH, 9q del
MDS	-5/5q del, -7/7q del, Trisomy 8, 11q del, 12p del, -13/13q del, 17p del/i(17q), 7q CN-LOH, 11q CN-LOH, 1p CN-LOH, 1q gain, Trisomy 21
Myeloid/lymphoid neoplasms with eosinophilia	4q12 del (FIP1L1-PDGFRA fusion)
B-ALL	-5/5q del, -7/7q del, Trisomy 8, 11q del, 12p del, -13/13q del, 17p del/i(17q), IKZF1 del (7p12.2), ERG del (21q22.2), CDKN2A/2B del (9p21.3), ETV6 del (12p13.2), PAX5 del (9p13.2), RB1 del (13q14.2)
T-ALL	TCR rearrangements with CNAs, 9q34.1 amp in NUP214-ABL1 fusion, 1p33 del in STIL-TAL1 fusion, 6q del, CDKN2A/2B biallelic del (9p21.3)
CLL	11q22.3 del (ATM and/or BIRC3), Trisomy 12, 13q14.2 del (MIR15A/16-1), 17p13.1 del (TP53), 2p12p25.3 gain (MYCN), 9p21.3 del (CDKN2A), Trisomy 19, 6q del, 14q24.1q32.3 del
MM	Trisomies of odd-numbered chromosomes, 1q21 gain, -17/17p13.1 del (TP53), 1p del, 14q del, 16q del
Burkitt-like lymphoma with 11q aberrations	11q CNAs

AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia/lymphoma; CLL, chronic lymphocytic leukemia; MM, multiple myeloma; del, deletion; dup, duplication; amp, amplification; CN-LOH, copy-neutral loss of heterozygosity; CNA, copy number abnormality.

(MDS), ALL, chronic lymphocytic leukemia (CLL), and multiple myeloma (MM) [11,16-21]. CMA application is recommended as the next step to detect novel findings such as small CNAs and CN-LOH, if normal results are obtained at the diagnosis or relapse of hematologic malignancies through karyotyping and FISH, CMA testing is also recommended as an alternative if the cell culture for karyotyping fails [10]. In ALL, some CNAs are indicative of gene fusions, such as 1p33 deletion (STIL-TAL1 fusion) and 9q34.1 amplification (NUP214-ABL1 fusion), which have diagnostic values (Table 2) [10,12].

LABORATORY STANDARDS AND QUALITY ASSURANCE OF CHROMOSOMAL MICROARRAY FOR HEMATOLOGIC MALIGNANCIES

Validation or verification of testing is required in the laboratory before clinical practice of CMA testing is conducted . During the validation process, the accuracy, precision, analytical sensitivity, specificity, and reportable range must be established. During the verification process applicable to Food and Drug Administration-approved tests, the accuracy, precision, and reportable range of results must be established using previously characterized samples. The percentage of abnormal cells was determined by a dilution study using samples with known copy number changes [13].

The laboratory must establish sample requirements and DNA quality thresholds. Generally, the primary recommended sample for hematologic malignancy is bone marrow (BM). A peripheral blood (PB) sample can be used as an alternative if malignant cells are sufficient in the PB [15]. The laboratory must establish thresholds for quality control (QC) metrics in assay procedures, such as DNA OD 260/280, quantity, and PCR product size requirements. The thresholds of data QC metrics, such as the median absolute pairwise difference (MAPD) and SNPQC, must be established and managed in the laboratory [7,13].

INTERPRETATION OF CMA RESULTS FOR HEMATOLOGIC MALIGNANCIES

The American College of Medical Genetics and Genomics (ACMG) and the Cancer Genomics Consortium (CGC) reported consensus recommendations regarding technical standards for the interpretation of CNAs and CN-LOH in neoplastic disorders. Interpretation of the CMA results for hematologic malignancies was based on a four-tier evidence-based categorization system. This system is similar to the sequence variant interpretation standards for somatic disorders and focused on the diagnostic, prognostic, and therapeutic significance. According to the evidence level, the CNAs or CN-LOH of the CMA results can be classified as Tier 1A/B (strong clinical significance), Tier 2 (some clinical significance), Tier 3 (clonal variants with no documented association with neoplastic disorder), and Tier 4 (benign or likely benign). Under special considerations, the germline pathogenic variants associated with cancer predisposition are classified as Tier 1A [12].

The interpretation of CMA results for hematologic malignancies can be highly dependent on other clinical information, such as clinical/pathologic diagnosis, and other test results, including karyotyping, FISH, and other molecular analyses. The same cytogenomic aberrations can be classified differently in different disorders.

Several public databases contain information on somatic copy number abnormalities. There is a lack of public data, except for the World Health Organization classification of hematolymphoid tumors. The laboratories are recommended to manage in-house databases to discriminate between significant and normal results. It is also recommended that laboratory standards be established to report incidental findings such as suspected germline variants associated with other clinical relevance such as constitutional disorders [12,22].

SUMMARY AND CONCLUSION

CMA is widely used in the diagnosis of hematologic malignancies such as AML, MDS, ALL, CLL, and MM. Although CMA has limitations in detecting balanced chromosomal rearrangements, it exhibits diagnostic utility for detecting small CNAs and CN-LOH. The CMA results for hematologic malignancies are clinically significant as diagnostic, prognostic, and therapeutic evidence. In Korea, healthcare reimbursements are necessary for the clinical application of CMA for hematologic malignancies. CMA testing is highly recommended to complement conventional karyotyping and FISH in various hematologic malignancies. An increase in CMA testing for hematologic malignancies is expected to provide novel diagnostic and prognostic findings for optimizing patient care and treatment.

CONFLICT OF INTEREST

I declare that I do not have any conflicts of interests.

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