Observational Study

Medicine®

OPEN

Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization for detecting chromosome abnormalities in myelodysplastic syndromes

A retrospective study

Xiaofei Ai, BS[a], Bing Li, MD[b], Zefeng Xu, MS[b], Jinqin Liu, MS[b], Tiejun Qin, BS[b], Qinghua Li, MD[a], Zhijian Xiao, MD[a,b,*]

Abstract

This study aimed to compare interphase fluorescence in situ hybridization (iFISH) and multiplex ligation dependent probe amplification (MLPA) for identifying genetic changes in myelodysplastic syndromes (MDS).

The frequencies of cytogenetic changes in MDS patients treated at the Institute of Hematology and Blood Disease Hospital (China) in 2009 to 2018 were assessed by iFISH based on bone marrow samples. Then, the effectiveness of MLPA in detecting these anomalies was evaluated.

Specimens from 287 MDS patients were assessed. A total of 36.9% (103/279) of MDS cases had chromosomal abnormalities detected by iFISH; meanwhile, 44.1% (123/279) harbored ≥1 copy-number variation (CNV) based on MLPA: +8 (n=46), −5 (n=39), −7 (n=27), del 20 (n=32) and del 17 (n=17). Overall, 0 to 4 aberrations/case were detected by MLPA, suggesting the heterogeneous and complex nature of MDS cytogenetics. There were 29 cases detected by MLPA, which were undetected by FISH or showed low signals. Sixteen of these cases had their risk classification changed due to MLPA detection, including 9 reassigned to the high-risk IPSS-R group. These findings demonstrated that MLPA is highly efficient in assessing cytogenetic anomalies, with data remarkably corroborating FISH findings (overall consistency of 97.1%). The sensitivities of MLPA in detecting +8, −5, −7, del 20 and del 17 were 92.3%, 97.1%, 100%, 100%, and 90%, respectively, with specificities of 95.8%, 97.6%, 97.7%, 97.6%, and 97%, respectively.

MLPA represents a reliable approach, with greater efficiency, accuracy, and speed than iFISH in identifying cytogenetic aberrations in MDS.

Abbreviations: CNAs = copy number aberrations, CNV = copy-number variation, iFISH = interphase fluorescence in situ hybridization, IPSS = the International Prognostic Scoring System, MDS = myelodysplastic syndromes, MLPA = multiplex ligation dependent probe amplification, .

Keywords: chromosomal abnormality, copy number aberration, fluorescence in situ hybridization, IPSS-R group, multiplex ligation-dependent probe amplification, myelodysplastic syndromes

Editor: Lydia Eccersley.

The present study was funded by the Tianjin Natural Science Funds under Grant (18JCZDJC34900 and 19JCQNJC09400), PUMC Youth Fund and Fundamental Research Funds for the Central Universities (No.3332019093), and State Key Laboratory of Experimental Hematolgy Research Grant (Z20-08).

The authors have no conflicts of interests to disclose.

Supplemental Digital Content is available for this article.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Department of Pathology, [b] MDS and MPN Centre, State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin, China.

[*] Correspondence: Zhijian Xiao, State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, 288 Nanjing Road, Tianjin 300020, China (e-mail: zjxiao@ihcams.ac.cn).

How to cite this article: Ai X, Li B, Xu Z, Liu J, Qin T, Li Q, Xiao Z. Multiplex ligation-dependent probe amplification and fluorescence in situ hybridization for detecting chromosome abnormalities in myelodysplastic syndromes: a retrospective study. Medicine 2021;100:18(e25768).

Received: 1 December 2020 / Received in final form: 7 April 2021 / Accepted: 10 April 2021

http://dx.doi.org/10.1097/MD.0000000000025768

1. Introduction

Myelodysplastic syndromes (MDS), heterogeneous myeloid clonal disorders that originate from hematopoietic stem cells, feature cytopenia and morphologic dysplasia, with substantial potential to develop into acute myeloid leukemia.[1–3] MDS occurs in 3–4/100,000 individuals in America, and incidence rises with age.[4] Establishing and developing efficient and rapid methods for cytogenetic and molecular analyses is a high priority, in order to comprehensively screen for disease-relevant copy number aberrations (CNAs) in clinical diagnosis.

Conventional cytogenetics constitutes the gold standard for defining the associations of gene loci with diseases, providing a complete gene profile and additional insights into the molecular bases of various diseases, and determining the involved gene loci. However, cytogenetic assessment is hindered by reduced mitotic activity in tumor cells in vitro, poor chromosome morphology, and substantial complexity. Interestingly, the broad genomic features of MDS have been assessed in large trials by fluorescence in situ hybridization (iFISH), single nucleotide polymorphism array, and next-generation sequencing.[5] For example, iFISH rapidly and reliably detects specific anomalies with direct prognostic implication such as del(5q) and an isolated del(7q), both of which, according to IPSS-R, confer a poor prognosis in MDS.[6,7] However, iFISH probes only detect predefined aberrations, and genetic anomalies beyond their coverage would be completely missed. In addition, iFISH is considered as a technique which targets single aberrations and is relatively costly, which can only detect deletions or amplifications of sequences >20 to 50 kb.[8]

Multiplex ligation-dependent probe amplification (MLPA) represents a multiplex PCR-based method originally designed for identifying CNAs.[9] MLPA probes interact with target sequences of 50 to 100 nucleotides, enabling its application for DNAs with high fragmentation.[10] Additionally, this assay can detect a small deletion retained in only 1 exon. Distinct MLPA panels have been proposed for specifically detecting multiple diseases, including genetic disorders and hematological malignancies, for example, multiple myeloma.[10,11]

Currently, studies comparatively examining iFISH and MLPA for their efficiencies in detecting MDS-related abnormalities are scarce. Therefore, the present study aimed to compare iFISH and MLPA for identifying genetic changes in MDS. In addition, we determined the feasibility for the diagnosis and risk-stratification of MDS by combining standard karyotype analysis, iFISH, and MLPA.

2. Materials and methods

2.1. Patients

This retrospective study assessed newly diagnosed patients with MDS at the Institute of Hematology and Blood Disease Hospital (China) between August 2009 and August 2018. Inclusion criteria were:

1. MDS diagnosis based on the 2016 WHO Classification standard[12];
2. bone marrow sample collected.

Exclusion criteria were:

1. no follow up;
2. incomplete test indicators;
3. not receiving standard treatment.

The present trial had approval by the ethics committee of the Blood Diseases Hospital, the Chinese Academy of Medical Sciences (LXKT2020004-EC-1), following the Declaration of Helsinki. Informed consent was not required for this retrospective study. The selected newly diagnosed MDS patients have been tested by iFISH related probes.

2.2. Interphase fluorescent in situ hybridization

Bone marrow mononuclear cells were assessed by iFISH. Briefly, multicolor probes were provided by Vysis laboratories (Abbott Laboratories, USA), including probes for −5/−5q, −7/−7q, +8, −20q, and 17p−. iFISH signals were evaluated under a fluorescence microscope (Olympus BX51, Japan) using the Q-FISH imaging software (IMSTAR, France). A total of 200 to 300 interphase nuclei were counted per slide. Positive threshold rates were based on a previous report.[13] The iFISH results were interpreted by 2 or more experts in molecular pathology, independently of parallel metaphase karyotype analysis.[5,8,10,11]

2.3. Multiplex ligation-dependent probe amplification

Bone marrow samples were obtained at diagnosis. Genomic DNA extraction was performed with AxyPrep Blood Genomic DNA Miniprep Kit (Axygen Biosciences, USA). The obtained DNA is stored in a −80°C freezer for later experimental research. A total of 50 ng gDNA was assessed by MLPA with SALSA MLPA P414-A1 MDS probemix (MRC-Holland, the Netherlands) containing 46 probes targeting the variable regions of the chromosome as well as 12 control probes targeting generally unchanged regions in MDS (Supplementary Table 1, http://links.lww.com/MD2/A138). MLPA reactions included quality and negative controls, and PCR products underwent assessment on an ABI 3130XL Genetic analyzer (Applied Biosystems) with the Coffalyser.net software (MRC Holland, the Netherlands) as instructed by the manufacturer.[14]

To rule out between-probe differences, normal ranges for respective MDS-specific probes were established for improving the accuracy of MLPA analysis. Additionally, DNA specimens isolated from peripheral blood obtained from 20 healthy donors underwent MLPA analysis to establish the probe-specific normal reference range. These 20 healthy donors were randomly selected from normal donors who donated peripheral blood stem cells. They have all undergone bone marrow examinations to ensure their health. The "mean±2SD" (95% confidence interval [CI], $P=.05$) and "mean±3SD" (95% CI, $P=.01$) values for various probes are shown in Supplementary Table 1, http://links.lww.com/MD2/A138. For data with large CIs, "mean±3SD" reference ranges served as respective cutoff values for CNV assessment.

2.4. Data collection and outcomes

In this study, the collected variables included age, sex, hemoglobin, absolute neutrophil count, platelet count, WHO 2016 class, and IPSS-R risk category.

Overall survival was measured from treatment initiation to the date of death or last follow-up based on the international uniform response criteria.[15]

Table 1

Characteristics of the MDS patients.

patients	Total (n = 279)
Age (years), median (range)	55 (25–83)
Male, n (%)	191 (68.5)
Hemoglobin (Hb; g/L), median (range)	79 (31–169)
Absolute neutrophil count (10^9/L), median (range)	1.1 (0.1–19.1)
Platelet count ($\times 10^9$/L), median (range)	69 (10–810)
WHO 2016 classification, n (%)	
MDS-SLD	6 (2.2)
MDS-MLD	139 (49.8)
MDS-RS	11 (3.9)
MDS-EB-1	58 (20.8)
MDS-EB-2	56 (20.1)
MDS-U	7 (2.5)
5q- syndrome	2 (0.7)
IPSS-R risk category, n (%)	
Very low	8 (2.8)
Low	68 (24.4)
Intermediate	85 (30.5)
High	64 (22.9)
Very high	54 (19.4)

IPSS = International Prognostic Scoring System.

2.5. Follow-up

Follow-up was performed at 3-month intervals by phone calls or outpatient visits, assessing survival, blood count, treatment, and review status. The last follow-up date was December 30, 2018.

2.6. Statistical analysis

SPSS v18.0 (SPSS Inc.) was employed for data analysis. The McNemar test was performed for comparing MLPA and iFISH in detecting genetic aberrations. Kaplan–Meier curves were generated for survival assessment, with the log-rank test carried out for comparison. $P < .05$ indicated statistical significance.

3. Results

3.1. Frequencies of genetic anomalies determined by iFISH and MLPA

A total of 287 MDS specimens underwent MLPA and iFISH analyses. Meanwhile, 8 cases were withdrawn due to insufficient amount of DNA retained, which made MLPA data unanalyzable. The characteristics of the 279 MDS patients assessed are shown in Table 1. A male-to-female ratio of 2.17/1 was found, and the median patient age was 55 (25–88) years. One hundred three (36.9%) MDS patients had chromosomal abnormalities in iFISH analysis, whereas 123 (44.1%) contained at least 1 CNV as determined by MLPA analysis. There were 0 to 4 genetic aberrations detected in individual patients (Fig. 1).

3.2. Abnormalities detected by MLPA

Chromosome 8 abnormalities (+8), including 8p and 8q amplifications, were found in 46/279 (16.5%) cases. NCOA2-5 was the most frequently detected 8q amplification (42/46), followed by MYC-3 and PTK2-33 (36/46). There were 26 patients with positive results for 8p amplification, all showing FGFR1-2; only 1 patient had 8p with the single probe deletion pattern.

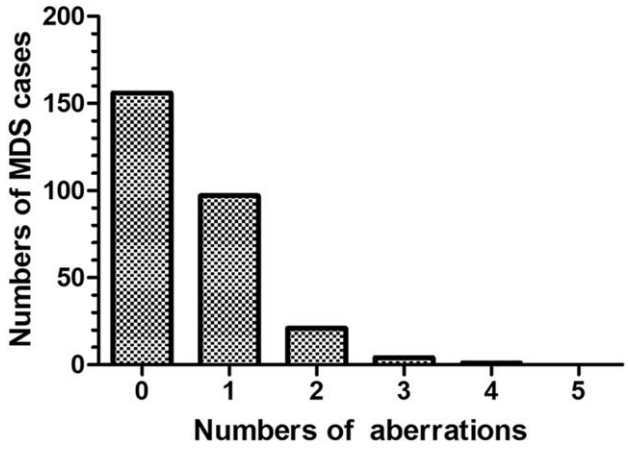

Figure 1. Distribution of MLPA-detected gene abnormalities.

Chromosome 5 abnormalities (−5), including 5p and 5q deletions, were detected in 39/279 cases (14.0%). EGR1-1 and EGR1-2 constituted the most frequent 5q deletion (38/39), followed by APC-18, MIR145-, SPARC-7, and MIR146A-1, respectively. One patient had chromosome 5p deletion with the single probe deletion pattern.

Chromosome 7 abnormalities (−7), including 7p and 7q amplifications, were found in 27/279 (9.7%) cases. CDK6-8 and EZH2-13 constituted the most frequent 7q deletion (21/27). Additionally, 9 patients showed positive results for 7q deletion with the single probe deletion pattern; in these individuals, IKZF1-3 was the most frequent 7p deletion (7/9).

Chromosome 20 abnormalities (20q deletions or del 20) were detected in 32/279 (11.5%) cases. ASXL1-4 was the most frequently detected 20q deletion (22/32).

Chromosome 17 abnormalities (del 17), including 17p and 17q deletions, were detected in 17/279 (6.1%) cases. There were 13 patients with positive results for 17p deletion, 5 positive for 17q deletion, and 1 harboring both 17p and 17q deletions. TP53-10 was the most frequent 17p deletion (12/13), and NF1-17 the most frequently detected 17q deletion (3/5).

3.3. MLPA vs conventional iFISH for detecting cytogenetic abnormalities

To assess MLPA performance in diagnosing CNVs in MDS, iFISH findings in 279 patients were retrospectively assessed, comparatively to MLPA data. The frequencies of +8, −5, −7, del 20 and del 17 determined by iFISH were 14.0% (n = 39), 12.2% (n = 34), 7.5% (n = 21), 9.3% (n = 26) and 3.6% (n = 10), respectively; these values for MLPA were 16.5% (n = 46), 14.0% (n = 39), 9.7% (n = 27), 11.5% (n = 32) and 6.1% (n = 17), respectively. Overall, the chromosome abnormality rate was higher in MLPA compared with iFISH, although the differences were non-significant (Table 2).

We further confirmed the consistency of data obtained from conventional iFISH and MLPA. The recurrent genetic alterations detected by iFISH and MLPA are summarized in Table 3 and Table S2 (Supplementary Table 2, http://links.lww.com/MD2/A138, respectively. Interestingly, iFISH determined 25 patients to be normal, while MLPA detected anomalies. In addition, 4 cases assessed with abnormalities by iFISH had more copy number

Table 2

Frequencies of genetic aberrations detected by iFISH and MLPA (n = 279).

	iFISH (%)	MLPA (%)
+8	14.0%(n = 39)	16.5%(n = 46)
−5	12.2%(n = 34)	14.0%(n = 39)
−7	7.5%(n = 21)	9.7%(n = 27)
Del 20	9.3%(n = 26)	11.5%(n = 32)
Del 17	3.6%(n = 10)	6.1%(n = 17)

Table 3

Abnormalities determined by iFISH but not detected by MLPA (n = 5).

	iFISH mosaic (%)	Target region (s) for gene (s)
Patient 112	10.1%	TP53
Patient 126	10.8%	D5s721, D5S23 and EGR1
Patient 146	8.4%	CEP8
Patient 147	4.8%	CEP8
Patient 149	6.2%	CEP8

changes determined by MLPA. Furthermore, 16/29 (55%) of patients assessed by MLPA detection as belonging to the IPSS - R group had their classification changed, including 9 cases re-categorized into a higher-risk IPSS-R group (Supplementary Table 2, http://links.lww.com/MD2/A138).

The results obtained by iFISH and MLPA in detecting +8, −5, −7, del(17) and del (20) were consistent in this study based on the McNemar test. The iFISH and MLPA showed significant differences for −7, del(17) and del (20) (Table 4). Out of 1395 (5 × 279) comparisons, 1354 results were concordant, indicating an overall consistency of 97.1%. MLPA's sensitivity and specificity in identifying +8 were 92.3% and 95.8%, respectively; MLPA detected −5 with sensitivity and specificity of 97.1% and 97.6%, respectively. Besides, MLPA's sensitivity and specificity in identifying −7 were 100% and 97.7%, respectively; these values were 100% and 97.6% for del (20), and 90.0% and 97.0% for del (17), respectively. These results further indicated the potential use of MLPA for detecting common genetic aberrations in MDS.

3.4. Prognostic values of the aberrations detected by MLPA

To investigate the prognostic values of chromosomal alterations identified by MLPA, the survival of 279 MDS patients (median follow-up of 15 months, ranging between 1 and 102 months) was examined. In this study, +8 abnormalities exhibited no prognostic value, likely because of small sample size and distinct therapeutic approaches. Meanwhile, patients with −5, −7, del (17), and del(20) had remarkably reduced OS (10.6 vs 81.8 months, $P < .0001$; 12.2 vs 80.6 months, $P < .0001$; 13.4 vs 80.2 months, $P < .0001$; and 41.1 vs70.2 months, $P = .0427$, respectively) compared with cases without these abnormalities (Supplementary Fig. 1, http://links.lww.com/MD2/A137).

4. Discussion

This study demonstrated that MLPA represents a reliable method, and is superior to iFISH in detecting cytogenetic abnormalities in MDS, with comparatively higher efficiency, increased accuracy and faster procedure.

Currently, several methods are applied for detecting CNAs such as standard chromosome analysis, iFISH, MLPA, and array-based techniques.[16] The karyotype constitutes a major constituent of the International Prognostic Scoring System (IPSS) and revised-IPSS, with an essential role in MDS prognosis.[17] Common cytogenetic abnormalities detected in newly diagnosed cases comprise −7/7q-, −5/5q-, +8, 20q−, −Y, i(17q) or t(17p), −13/13q−, 11q−, and 12p− or t(12p); the most frequent aberrations, that is, −7/7q−, −5/5q−, +8 and 20q−, are found in about 40% of MDS patients. The current gold standard for cytogenetic diagnosis in MDS is conventional karyotyping of banded metaphase chromosomes in the bone marrow.[18] To date, iFISH application attracts increasing attention in cytogenetics due to high resolution and great success rate.[17] Nevertheless, iFISH probes are expensive and cover ~20 kb at best; consequently, only large and very frequent aberrations are identified by iFISH.[19,20]

MLPA allows the detection of targeted CNVs in multiple human genes concurrently.[21] Here, MLPA was performed to screen CNVs showing tight associations with MDS prognosis as previously reported. In general, signals are regarded as aberrant because cut-off values are below 0.7 (deletion) and above 1.3 (duplication/ amplification).[22]

The value of MLPA in assessing acute leukemia and myelodysplastic diseases has been investigated, demonstrating its outstanding sensitivity and specificity based on iFISH. In MLPA, the reaction mixture comprises probes for chromosomes 5,7,8,11,12 (ETV6), 17 (TP53), 20, and 21 (RUNX1). Recently, Wang and collaborators performed MLPA for detecting CNVs in 437 individuals with MDS, revealing 35% of them with 1 or more CNVs; compared with R-banding karyotyping, approximately 45% of these MDS cases showed chromosomal aberrations. This study firstly evaluated the utilization of specific ranges for various probes in MDS patients by MLPA. The frequencies of aberrations in the current cohort were consistent with previous reports.[23,24]

Table 4

Sensitivities and specificities of MLPA based on iFISH as the gold standard.

FISH		+8		-5		-7		del 20		del 17	
		+	−	+	−	+	−	+	−	+	−
MLPA	+	36	10	33	6	21	6	26	6	9	8
	−	3	230	1	239	0	252	0	247	1	261
McNemar test		$P = .092$		$P = .125$		$P = .031$		$P = .031$		$P = .039$	

It is well-known that iFISH is applied for MDS detection as a supplement of conventional cytogenetics. Meanwhile, this study showed remarkable agreement between iFISH and MLPA, although there were some discrepancies. As shown above, the highest discordance was found in +8, with CEP8 as the most frequent genetic aberration.

These discrepancies may be attributed to distinct probes employed in both methods, which might result in differences in resolution, point mutations and subclones. First, iFISH can only identify deletions or amplifications of sequences >20 to 50 kb, while MLPA recognizes 50 to 100-nt sequences. Therefore, MLPA could detect highly fragmented recessive lesions.[25] Secondly, a mutation/polymorphism detected by a probe could also reduce the related peak area in the MLPA method, whose detection is impossible by iFISH. Generally, multiple MLPA probes are used for detecting a given gene or distinct genes in a certain chromosome region, which increases the reliability of MLPA.[26] Thirdly, with new technologies, for example, CGH and next-generation sequencing, clonal heterogeneity, and evolution have emerged as critical concepts in the field of oncology, especially in MDS pathogenesis.

However, MLPA assessment of cancer specimens provides insights into the total cells. In case a particular genetic anomaly is found only in a minute subclone, its detection is unlikely. In contrast, aberrations found in all single tumor cells are identified by iFISH. This shortcoming of MLPA indicates the irreplaceability of iFISH. Jointly, the above findings suggest that MLPA could identify CNVs with high throughput and elevated resolution, while iFISH identifies both balanced and unbalanced aberrations with higher reliability in detecting gene alterations in minute subclones. MLPA and iFISH therefore have mutual complementarity for detecting cytogenetic abnormalities in MDS.

The major similarities between MLPA and iFISH are that both techniques use bone marrow nucleated cells, and include 5-minute degeneration and 16 to 20-hour hybridization. The differences are as follows. In MLPA, mononuclear cells are used for DNA extraction, with a DNA concentration of 20 to 30 ng/μL. Meanwhile, iFISH is performed by fixing the interstitial cell dispersion area after hypotonic treatment of white blood cells; 500 interstitial cells are counted, determining the percentage of positive signals. The sensitivity of iFISH can reach 10^{-3}, while that of MLPA reaches 10^{-4} to 10^{-5}. However, FISH probes have relatively low resolution (~20 kb at best). In addition, novel mutations are undetectable by iFISH. Consequently, revealing all lesions associated with prognosis in MDS is critical. MLPA has strong specificity and can detect the change of mononucleotide in the sequence.MLPA assesses up to 50 CNVs in the same PCR reaction, and kits can be readily updated based on most recent cytogenetic findings. For this reason, MLPA is considered an effective and robust diagnostic tool in multiple disorders.[25]

In practice, MLPA has more advantages than iFISH. In MLPA, each reaction only needs to be performed in the same test tube, and 30-50 target sequences can be amplified simultaneously. However, the iFISH hybridization process can only evaluate 12 samples at a time, with up to 36 probes, and each sample could only detect 1 chromosome abnormality. In terms of cost, MLPA is also relatively superior to iFISH. In China, FISH is very costly. For example, + 8 and del20 probes, which are generally monochromatic, cost about 400 Yuan/probe. Other probes, for example, –7 and –5, which are dichromatic, cost 700 Yuan/probe. Meanwhile, the cost of MLPA reagents is low for all the reagents needed for the reaction are included in the kit, and the cost of a single signal is only 5 Yuan. However, the cost of the MDS-related combinatory FISH probe is much higher than that of MLPA. Besides, FISH tests usually include only commonly detected sequences. As for MLPA, it can be routinely used for new patients, also applied to complex karyotypes. But MLPA in this work could not detect balanced translocations or generate a complete MLPA profile in a case with low amounts of tumor cells, indicating the limitations of this technique.

5. Conclusion

Compared to iFISH as the current gold standard for detecting cytogenetic abnormalities in MDS, MLPA is sensitive, simple, and amenable to increased multiplex. Its main advantages vs cytogenetics-based techniques are that MLPA has an easier procedure, enhanced resolution, and higher throughput. Therefore, MLPA represents an ideal screening technique, and could be used as a complementary method to iFISH.

Author contributions

Conceptualization: Xiaofei Ai, Zhijian Xiao.
Data curation: Xiaofei Ai, Bing Li, Zefeng Xu, Jinqin Liu, Tiejun Qin.
Formal analysis: Xiaofei Ai, Bing Li, Zefeng Xu, Jinqin Liu.
Funding acquisition: Bing Li, Zefeng Xu, Jinqin Liu.
Project administration: Zhijian Xiao.
Resources: Tiejun Qin, Qinghua Li.
Writing – original draft: Xiaofei Ai.
Writing – review & editing: Qinghua Li, Zhijian Xiao.

References

[1] Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol 1982;51:189–99.
[2] Swerdlow SH, Campo E, Harris NL. WHO classifification of tumours of haematopoietic and lymphoid tissues. Lyons: France IARC Press 2017;106–20.
[3] Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood 2016;127:2391–405.
[4] Montalban-Bravo G, Garcia-Manero G. Myelodysplastic syndromes: 2018 update on diagnosis, risk-stratification and management. Am J Hematol 2018;93:129–47.
[5] Song Q, Peng M, Chu Y, et al. Techniques for detecting chromosomal aberrations in myelodysplastic syndromes. Oncotarget 2017;8: 62716–29.
[6] An G, Yan Y, Xu Y, et al. Monitoring the cytogenetic architecture of minimal residual plasma cells indicates therapy-induced clonal selection in multiple myeloma. Leukemia 2020;34:578–88.
[7] Vardiman J, Reichard K. Acute myeloid leukemia with myelodysplasia-related changes. Am J Clin Pathol 2015;144:29–43.
[8] He R, Wiktor AE, Durnick DK, et al. Bone marrow conventional karyotyping and fluorescence in situ hybridization: defining an effective utilization strategy for evaluation of myelodysplastic syndromes. Am J Clin Pathol 2016;146:86–94.
[9] Thakral D, Kaur G, Gupta R, et al. Rapid identification of key copy number alterations in B- and T-cell acute lymphoblastic leukemia by digital multiplex ligation-dependent probe amplification. Front Oncol 2019;9:871.
[10] Stuppia L, Antonucci I, Palka G, et al. Use of the MLPA assay in the molecular diagnosis of gene copy number alterations in human genetic diseases. Int J Mol Sci 2012;13:3245–76.
[11] Hömig-Hölzel C, Savola S. Multiplex ligation-dependent probe amplification (MLPA) in tumor diagnostics and prognostics. Diagn Mol Pathol 2012;21:189–206.

[12] Hong M, He G. The 2016 eevision to the World Health Organization classification of myelodysplastic syndromes. J Transl Int Med 2017;5:139–43.
[13] Lai YY, Huang XJ, Li J, et al. Standardized fluorescence in situ hybridization testing based on an appropriate panel of probes more effectively identifies common cytogenetic abnormalities in myelodysplastic syndromes than conventional cytogenetic analysis: a multicenter prospective study of 2302 patients in China. Leuk Res 2015;39:530–5.
[14] de Boer S, White SJ. Genotyping multiallelic copy number variation with multiplex ligation-dependent probe amplification (MLPA). Methods Mol Biol 2017;1492:147–53.
[15] Anderson KC, Kyle RA, Rajkumar SV, et al. Clinically relevant end points and new drug approvals for myeloma. Leukemia 2008;22:231–9.
[16] Volkert S, Haferlach T, Holzwarth J, et al. Array CGH identifies copy number changes in 11% of 520 MDS patients with normal karyotype and uncovers prognostically relevant deletions. Leukemia 2016;30:257–60.
[17] Mohamedali AM, Gäken J, Ahmed M, et al. High concordance of genomic and cytogenetic aberrations between peripheral blood and bone marrow in myelodysplastic syndrome (MDS). Leukemia 2015;29:1928–38.
[18] de Swart L, Smith A, Haase D, et al. Prognostic impact of a suboptimal number of analyzed metaphases in normal karyotype lower-risk MDS. Leuk Res 2018;67:21–6.
[19] Kokate P, Dalvi R, Koppaka N, et al. Prognostic classification of MDS is improved by the inclusion of FISH panel testing with conventional cytogenetics. Cancer Genet 2017;216-217:120–7.
[20] Merkel D, Soffer S, Novikov I, et al. Is fluorescence in-situ hybridization sufficient in patients with myelodysplastic syndromes and insufficient cytogenetic testing? Leuk Lymphoma 2019;60:764–71.
[21] Alhourani E, Rincic M, Othman MA, et al. Comprehensive chronic lymphocytic leukemia diagnostics by combined multiplex ligation dependent probe amplification (MLPA) and interphase fluorescence in situ hybridization (iFISH). Mol Cytogenet 2014;7:79.
[22] Eijk-Van Os PG, Schouten JP. Multiplex Ligation-dependent Probe Amplification (MLPA) for the detection of copy number variation in genomic sequences. Methods Mol Biol 2011;688:97–126.
[23] Wang J, Ai X, Qin T, et al. Multiplex ligation-dependent probe amplification assay identifies additional copy number changes compared with R-band karyotype and provide more accuracy prognostic information in myelodysplastic syndromes. Oncotarget 2017;8:1603–12.
[24] Donahue AC, Abdool AK, Gaur R, et al. Multiplex ligation-dependent probe amplification for detection of chromosomal abnormalities in myelodysplastic syndrome and acute myeloid leukemia. Leuk Res 2011;35:1477–83.
[25] Zang M, Zou D, Yu Z, et al. Detection of recurrent cytogenetic aberrations in multiple myeloma: a comparison between MLPA and iFISH. Oncotarget 2015;6:34276–87.
[26] Ooi A, Oyama T, Nakamura R, et al. Semi-comprehensive analysis of gene amplification in gastric cancers using multiplex ligation-dependent probe amplification and fluorescence in situ hybridization. Mod Pathol 2015;28:861–71.