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ORIGINAL ARTICLE

Deciphering the complexities of *MECOM* rearrangement-driven chromosomal aberrations

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Abstract

MECOM rearrangement is associated with rapid disease progression and poor prognosis in myeloid neoplasms. Previous studies were often based on 3q26.2 abnormalities without confirmation of *MECOM* status. The frequency of *MECOM* rearrangement and attribution of various chromosomal aberrations remain poorly characterized. This study presented 129 cases with confirmed *MECOM* rearrangement by karyotyping and multiple FISH methodologies. *MECOM* rearrangement arose through translocation (49.6%, $n=64$), inversion (40.3%, $n=52$), insertion (5.4%, $n=7$) or unknown mechanism(s) (4.7%, $n=6$). The classic inv(3)(q21q26.2) was dominant ($n=50$) in inversion-driven *MECOM* rearrangement; and 3 of them also had double inv(3). For translocation-driven *MECOM* rearrangement, t(3;21) was most common ($n=15$), followed by *t*(2;3) ($n=13$), t(3;12) ($n=10$), t(3;3) ($n=9$), t(3;8) ($n=6$), t(3;6) and t(3;17) ($n=4$ each), t(1;3) and t(3;?) ($n=1$ each). Cases with t(3;3)-, t(3;12)-, and insertion-driven *MECOM* rearrangement were prone to exhibit a complex karyotype, while cases with t(2;3)-, t(3;21)- and insertion-driven *MECOM* rearrangement were prone to have an "unbalanced" MECOM FISH signal pattern, likely caused by uncommon breakpoint(s) within the target of *5'MECOM* probe. Therefore, atypical chromosomal aberrations and/or mechanisms are involved in *MECOM* rearrangement. Confirmation/exclusion of *MECOM* rearrangement is necessary in all cases with a 3q26.2 abnormality. (Word count: 190)

Keywords *MECOM* rearrangement, Myeloid neoplasms, Karyotyping, Fluorescence in situ hybridization (FISH), Map-back, aCGH.

Introduction

In the 2017 World Health Organization classification of tumors of hematopoietic and lymphoid tissues (2017 WHO), acute myeloid leukemia associated with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) is classified as its own entity [1], emphasizing the unique clinicopathologic features and poorer prognosis of this subgroup of AML patients [2–5]. The underlying mechanism is characterized by dysregulated expression of MDS1 and EVI1 complex locus (MECOM) at chromosome 3q26.2 and GATA Binding Protein 2 (GATA2) at chromosome 3q21 [6,7]. However, inv(3)(q21q26.2) and t(3;3)(q21;q26.2) are also found in other myeloid neoplasms, such as myelodysplastic syndrome (MDS) [8–10], blast phase of chronic myeloid leukemia (CML) [11–18] and classical Philadelphia chromosome-negative myeloproliferative neoplasms (Ph− MPNs) [19,20]. Most patients with these diseases exhibit rapid disease progression, worse response to chemotherapy and/or tyrosine kinase inhibitors (TKIs) therapy, and a shorter survival than patients with same

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disease(s) but without inv(3)(q21q26.2)/t(3;3)(q21;q26.2) [7–19]. A variety of other chromosomal aberrations with involvement of 3q26.2, such as t(2;3) [21]; t(3;8) [22-24]; t(3;12) [25], and t(3;21) [26,27], also have been reported in almost all types of myeloid malignancies with clinicopathologic features comparable to those with inv(3)(q21q26.2)/t(3;3)(q21;q26.2). Therefore, others have suggested [2–4,19,22,27,28] that all cases with 3q26.2/*MECOM* rearrangement, regardless of the morphologic features, should be categorized as a separate entity. Taken together, a variety of chromosomal aberrations involving 3q26.2, including inv(3)(q21q26.2) and t(3;3)(q21;q26.2), are found in different myeloid malignancies. These 3q26.2 abnormalities are usually considered as a poor prognostic marker.

Most previous studies performed on this topic have included only a limited number of cases and/or only one type of 3q26.2 abnormality. In other studies, chromosomal analysis results indicating a 3q26 involvement (not 3q26.2 due to low resolution of chromosomal analysis) has been reported, but without a further confirmation of *MECOM* rearrangement. In 2015, De Braekeleer et al. [28] summarized a total of 1038 myeloid malignancies with 3q26.2 involvement reported in literatures and in public databases, such as PubMed (https://www.ncbi.nlm.nih.gov/pubmed/), the Mitelman Database of Chromosome Aberrations and Gene Fusions in Cancer (https://cgap.nci.nih.gov/Chromosomes/Mitelman) and the Atlas of Genetics and Cytogenetics in Oncology and Hematology (http://AtlasGeneticsOncology.org). Among 111 different chromosomal abnormalities involving 3q26.2 identified in these reports, in only 34 cases was *MECOM* rearrangement confirmed by fluorescence in situ hybridization (FISH) and/or other molecular techniques (e.g., Southern-blot and RT-PCR). Therefore, the prevalence of *MECOM* rearrangement (not only 3q26.2 aberrations) and attribution of various mechanisms causing *MECOM* rearrangement remain under-characterized.

In this study, we report 129 patients with myeloid neoplasms associated with FISH-proven *MECOM* rearrangement. The primary goal of this study is to more fully characterize the complexity of chromosomal aberrations involving 3q26.2/*MECOM* rearrangement in myeloid malignancies.

Materials and methods

Study group

In May of 2009, the Clinical Cytogenetic Laboratory at The University of Texas MD Anderson Cancer Center (MDACC) validated a MECOM FISH test for clinical use. This test is usually performed as an add-on to conventional cytogenetics analysis (karyotyping) to confirm or exclude a 3q26.2/*MECOM* involvement, or as a follow-up test for patients who tested positive previously for *MECOM* rearrangement. To date, approximately 350 MECOM FISH tests have been performed. We searched the cytogenetics database for MECOM FISH positive cases from May 1, 2009 to August 15, 2018. Cases with 3q26 abnormality by conventional cytogenetics but negative for and/or not been confirmed for *MECOM* rearrangement are excluded from this study. Clinicopathologic data, including *MECOM* FISH test results, were collected by electronic medical chart review. This study was approved by the MDACC Institutional Review Board and conducted in accordance with the Declaration of Helsinki.

Karyotype analysis

Conventional G-banded chromosomal analysis was performed on unstimulated 24-h and 48-h BM aspirate cultures using standard techniques as reported previously [24,29,30]. Routinely, 20 metaphases are analyzed by two technologists and the results are reported following the 2016 International System for Human Cytogenetics Nomenclature (ISCN 2016) guidelines. A complex karyotype is defined as $\geq$3 chromosomal abnormalities, of which at least one structural chromosomal abnormality is present [29].

Fluorescence in situ hybridization (FISH) analysis

The *MECOM* (*EVI1*) dual color, breakapart (BAP) DNA probe (#KI-10204) from Leica Biosystems/Kreatech (Buffalo Grove, IL) was used for all cases in this study. This probe hybridizes to band 3q26.2 (green on the centromeric side and red on the telomeric side of the *MECOM* gene) (Fig. 1). The Vysis LSI *BCR/ABL* ES Dual Color Fusion probe, Vysis *MYC* BAP probe, Vysis *ETV6* BAP Probe and Vysis *RUNX1T1/RUNX1* Fusion probe (Abbott Molecular, Des Plaines, IL) were also employed to assess a subset of cases in this study. All probes used for clinical diagnosis were validated in our laboratory in accord with the American College of Medical Genetics and Genomics (ACMGG) guidelines. The normal cutoff values for the *MECOM* probe established at 95% confidence level ($P < 0.05$) are the following: 6.0% for a signal pattern of 1R1G1F; 2.5% for 1R2F; 2.1% for 1G2F; 2.6% for 1F and 3.7% for 3F. The signal patterns of 1R1G1F, 1R2F and 1G2F are all indicative of *MECOM* rearrangement, whereas 1F and 3F usually mean loss (1F) or gain (3F) of one extra copy of *MECOM*.

Interphase FISH analysis was performed on a 24-hour culture of a BM aspirate specimen, and routinely 200 nuclei for each case are analyzed by two technologists. For some atypical cases, a map-back FISH was performed on previously G-banded and karyotyped metaphases to define both the signal pattern(s) and the location of each signal. Briefly, the same slide used for karyotype analysis was stripped of Geimsa stain and then hybridized with the *MECOM* BAP probe. Following the coordinates recorded by metaphase finder/capture system, the same metaphases used karyotype analysis were analyzed and documented for probe localization and signal pattern(s). The Aquarius Whole Chromosome Painting (WCP) probes for chromosomes 3 and 21 (Cytocell, Tarrytown, NY) were used for metaphase FISH in two cases. Both map-back FISH and WCP were performed by following standard protocols as reported previously [30].

Array comparative genomic hybridization (aCGH) analysis

As reported previously, a customized aCGH targeting cancer genes related to hematologic malignancies (4×180 K format; Agilent Technologies, Santa Clara, CA) was performed on a

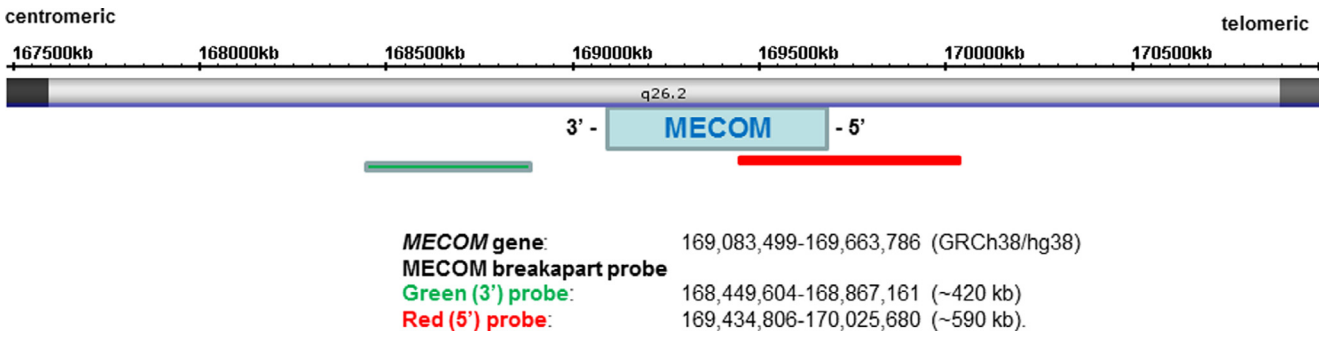

Fig. 1 Schematic representation of the *MECOM* gene located on 3q26.2 and target/size of *MECOM* breakapart probe used in this study. The nucleotide (nt) coordinates of the proximal green *3'* probe and the distal red 5*'* probe are kindly provided by Leica Biosystems.

subset of the study group at the request of the patient's clinician [30]. The analytical sensitivity (lower limit of detection) in a given sample is approximately 1 in 5 (20%) aberration-containing cells. The average resolution of this assay is about 1 Mb, but can reach to 25 kb for certain regions of interest, such as the *MECOM, MYC* and *ETV6* loci [29–31] in this study.

Results

Study group

The cohort included 129 patients with at least one positive MECOM FISH result. There were 73 men and 56 women with a median age of 57 years (range, 17–91 years) at the time of first detection of *MECOM* rearrangement. Except one patient (case# 129) with a plasma cell neoplasm, all the other patients had a myeloid neoplasm of various types, including acute myeloid leukemia (AML) ($n=77$; including both *de novo* AML and therapy-related AML and 1 case with donor cell AML); myelodysplastic syndrome (MDS) ($n=38$; including both *de novo* and therapy-related MDS); chronic myeloid leukemia (CML) ($n=11$, including blast phase, $n=8$ and chronic phase, $n=3$), chronic myelomonocytic leukemia (CMML) ($n=1$) and polycythemia vera with grade 3 myelofibrosis (MF-3) ($n=1$) (Table 1 and see [37]). Many patients (27.1%; 35/129) had a history of other malignancies, such as prostate cancer, breast cancer, multiple myeloma, as well as others. The patient with a plasma cell neoplasm had received chemotherapy and later stem cell transplant (SCT), but her most recent BM evaluation showed evidence of MDS. During a follow up interval ranging from 0 to 70 months (mean: 8 months), 89 patients died. There were 40 patients alive at last follow up including 5 in complete remission, 23 in partial remission, and 12 patients without remission. Four out of the 5 patients with CR had received SCT.

Characterization of *MECOM* rearrangements by chromosomal analysis

Except one patient (case# 128) in whom karyotype analysis could not be performed due to an extreme hypocellularity of the BM specimen and rapid demise of the patient, all other patients had at least one karyotype performed in this study. This study showed a complex karyotype in 61 (48%) patients, isolated 3q aberration in 33 (25.6%) patients, and 3q aberration with one additional chromosomal aberration in 34 (26.4%) patients (Table 1). The most common additional chromosomal aberration was monosomy7 (−7) and/or 7q deletion (7q-) in 51 (39.5%) patients.

In total, 64 cases showed *MECOM* rearrangement through translocations. The most common translocation was t(3;21) in 15 cases, followed by t(2;3) in 13, t(3;12) in 10; t(3;3) in 9; t(3;8) in 6; t(3;6) in 4; t(3;17) in 4, and t(1;3) in 1 case, respectively. Other mechanisms of *MECOM* rearrangement included inversions in 52 (40.3%) cases; insertions in 7 (5.4%) cases and the mechanisms were unknown in 6 cases (4.7%). Almost 50% cases in this group exhibited a complex karyotype, especially in cases with t(3;3) (6 of 9) and t(2;3) (7 of 13). The −7/7q- was more common in cases with t(3;3) (88.9%, 8/9) and t(3;12) (50%, 5/10) than cases with other translocations (Table 1).

Although the frequency of inversions of chromosome 3 (40.3%, 52/129), or briefly inv(3), is less than the translocations mentioned above in this study, it is much higher than each individual translocation. Several very interesting phenomenon were observed in the group of cases with inv(3): (1) although most ($n=50$) of these cases had a classic paracentric inv(3)(q21q26.2), 3 cases exhibited an inv(3) with different breakpoints other than 3q21, but all with confirmed *MECOM* rearrangement (cases #1, #2, and #47); (2) three cases (cases #44-#46) had double inv(3) affecting both chromosomes 3 [31], the first two with rearrangement of both *MECOM* genes and case #46 with rearrangement of only one *MECOM* gene. It is uncertain whether the inv(3)(p21q28) in this case was a constitutional abnormality without causing *MECOM* rearrangement; (3) five cases (cases #48-#52) exhibited an atypical structural aberration of the affected chromosome 3 in addition to the inv(3) which caused the *MECOM* rearrangement; and (4) the inv(3) group presented a slight lower frequency of complex karyotypes (44.2%, 23/52 vs. 47.3%, 61/129) but a higher frequency of −7/7q- (42.3%, 22/52 vs. 39.5%, 51/129) than the averages in this study.

Seven cases (cases #116-#121) had a *MECOM* rearrangement that occurred via insertion of a DNA segment containing partial *MECOM* gene/locus into different recipient chromosomes, e.g., chromosome 1, 3, 8 or even unidentifiable chromosome(s). Five cases (71.4%, 5/7) had a complex karyotype and 3 of them (42.9%, 3/7) with −7/7q- were in this group.

Table 1 The results of conventional cytogenetics and FISH analyses.

General information
Total 129 cases; 73M/56F
Age: median 57 y (range: 17–91 y)
Survival/followup: average 8 m (range: 0–70 m)
Outcome: D 89, CR 5, PR 23, NR 12
Diseases: AML 77, MDS 38, CML 11, others 3)

MECOM rearrangement	Total (%)	Karyotype results				MECOM FISH signal pattern (s)	
		Complex (%)	3q + ACA (%)	Isolated 3q (%)	7q-/−7 (%)	Balanced (%)	"Unbalanced" (%)
Inversion	52 (40.3)	23 (44.2)	15 (28.8)	14 (26.9)	22 (42.3)	48 (92.3)	4 (7.7)
Translocation	64 (49.6)	31 (48.4)	16 (25%)	17 (26.6)	25 (39.1)	43 (67.2)	21(32.8)
t(1;3)	1	0	0	1	0	1	0
t(2;3)	13	7	3	3	2	5	8
t(3;3)	9	6	2	1	8	8	1
t(3;6)	4	1	0	3	1	3	1
t(3;8)	6	3	0	3	3	5	1
t(3;10;21)	1	0	0	1	0	0	1
t(3;12)	10	5	2	3	5	9	1
t(3;17)	4	2	2	0	1	4	0
t(3;21)	15	6	7	2	4	8	7
t(3;?)[b]	1	1	0	0	1	0	1
Insertion	7 (5.4)	5 (71.4)	1 (14.3)	1 (14.3)	3 (42.9)	3 (42.8)	2 (67.2)
Others/unknown	6[a] (4.7)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	3 (50)	3 (50)
Total	129 (100)	61 (47.3)	34 (26.4)	33 (25.6)	51 (39.5%)	97 (75.2)	32 (24.8)

M: male; F: female; y: years; m: months.
ACA: additional chromosomal aberration; D: deceased; A: alive; CR: complete remission; PR: partial remission; NR: no response.
[a] One case was not karyotyped.
[b] A partner chromosome is unidentified based on chromosomal and map-back FISH analyses.

The mechanism(s) for *MECOM* rearrangement remains unknown in 6 cases (cases #123–#128) in this study. Although case #125 had an apparent add(3)(q21), additional chromosomal material of unknown origin at 3q21, FISH tests showed a partial deletion of the 5′*MECOM* gene, possibly implying that either an insertion of 3′*MECOM* at 3q21 or inv(3)(q21q26.2) with a subsequent del(3)(q21) through add(3)(q21). Case #126 exhibited a del(3)(q21q25), but with an intact *MECOM* on this abnormal chromosome 3 confirmed by map-back FISH test. The low level of *MECOM* rearrangement detected by interphase FISH test was possibly a result from a cryptic chromosomal abnormality. Case #127 had an origin-unidentifiable ring chromosome with positive FISH signals for *MECOM* rearrangement. The remaining two cases (cases # 123 and #124) harbored morphologically normal chromosomes 3, but interphase FISH test was performed due to a positive outside history and demonstrated a positive *MECOM* rearrangement at a level of approximately 20%.

Characterization of *MECOM* rearrangement by FISH analysis

The resolution of karyotypes was very low in many cases in this study and the structural abnormalities involving chromosome(s) 3 were difficult to be further characterized by karyotype analysis only. Therefore, analysis of *MECOM* by FISH was applied to confirm or exclude a *MECOM* rearrangement. In 75 cases (58.1%), MECOM FISH test was performed on the previously G-banded and karyotyped slides (map-back FISH) to help better define the structural abnormalities on chromosomes 3 and other related chromosomes, as well as to precisely to locate each FISH signal. According to the *MECOM* breakapart probe design/coverage information provided by the manufacturer (Fig. 1), the 3′*MECOM* probe (green color) targets the centromeric flank region and 5′*MECOM* probe (red color) targets part of the 5′*MECOM* locus and its' flanking region. The gap of about 570 kb between the 3′ and 5′*MECOM* probes is considered as the main breakpoint cluster region (BCR) [32,33]. Two major types of interphase *MECOM* FISH signal patterns were defined in this study: "balanced" *MECOM* rearrangement with a clear split pattern of one-red (*5′MECOM*), one-green (*3′MECOM*) and one-fusion (intact *MECOM*) (1R1G1F) or two-red and two-green (2R2G; indicating that both copies of *MECOM* gene are rearranged)($n = 97$); and "unbalanced" *MECOM* rearrangement with one or more isolated red (*5′MECOM*) or green (*3′MECOM*) ($\geq$1R or $\geq$1G) signals (24.8%, 32/129) (Fig. 2 and Table 1). The "balanced" *MECOM* rearrangement signal pattern was most likely generated through a breakpoint within the BCR mentioned above. Among all cases with "unbalanced" *MECOM* rearrangement signal patterns, 26 (81.2%) exhibited a signal pattern of one-red and two fusion (1R2F) by interphase FISH. This "unbalanced 1R2F" signal pattern hasn't been observed so often during the *MECOM* BAP probe validation procedures and generated initially a confusion to our technologists who were performing the test on clinical specimen. Correlating with their map-back FISH results available in many cases (see [37]), the isolated red signal (*5′MECOM*) was found on the partner/recipient chromosome(s) and a fusion signal on the donor chromosome 3, implying that this type of "unbalanced" *MECOM* rearrangement signal pattern

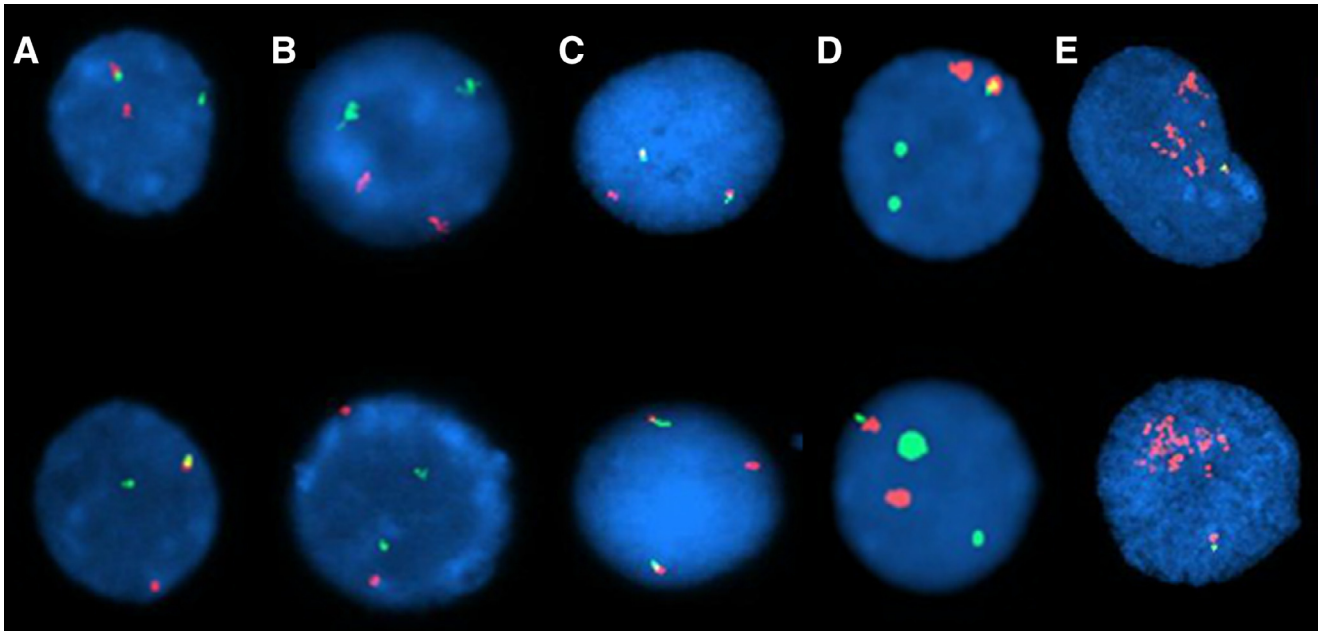

Fig. 2 The "balanced" and "unbalanced" interphase MECOM FISH signal patterns observed in this study. (A) A "balanced" signal pattern of 1R1G1F; (B) a "balanced" signal pattern of 2R2G; (C) an "unbalanced" signal pattern of 1R2F; (D) an "unbalanced" signal pattern of 1R2G1F; and (E) an "unbalanced" signal pattern of ampR1F.

was most likely generated by a breakpoint within the target of *5′ MECOM* probe (Fig. 1). The "1R2F" signal pattern also can be reported as "1R1rG1F" as suggested by the vendor for the same mechanism for breakpoints. Six cases (cases #48, #54, #82, #116, #122 and #126) presented atypical, most likely truly unbalanced *MECOM* rearrangement signal patterns in this study. Interestingly, cases with t(2;3) (8/13; 61.5%) [32], t(3;21) (7/15; 46.7%) [34], insertion (4/7; 57.1%) and unknown mechanism(s) (3/6; 50%) had a higher frequency of "unbalanced" MECOM rearrangement signal pattern(s) than all other groups/subgroups (Table 1).

Three cases (cases #44, #45 and #127) exhibited a signal pattern of 2R2G. Cases #44 and #45 had double inv(3)(q21q26.2) with rearrangement of both *MECOM* genes and case #127 had an un-identified ring chromosome with a 2R2G signal pattern on it (Fig. 2(B)). In addition to *MECOM* rearrangement, 2 cases exhibited amplification of *5′MECOM* (case# 122) (Fig. 2(E)) or intact *MECOM* (case #129) simultaneously.

All cases with t(9;22) were confirmed to be positive for *BCR/ABL1* by FISH test in this study. Two cases (cases #83 and #84) with t(3;8) were positive for *MYC* rearrangement and the 3′ *MYC* was translocated on the abnormal chromosome 3 as demonstrated by MYC FISH, indicating a simultaneous *MYC* and *MECOM* rearrangement, or likely a *MECOM/MYC* fusion (Fig. 3). Five cases with t(3;12) (cases #87, #88, #91, #92 and #95) and 1 case with ins(12;3) (case #120) were also positive for *ETV6* rearrangement confirmed by FISH. The *5′ETV6* signal was located on the abnormal chromosome 3 in two cases (cases #91 and #92) confirmed by map-back FISH, indicating a simultaneous *ETV6* and *MECOM* rearrangement, or likely an *MECOM/ETV6* fusion. Interestingly, the case with ins(12;3) (case #120) exhibited an *ETV6* rearrangement with a partial deletion of *5′ETV6*. Five cases with t(3;21) (cases #105, #106, #110, #111 and #114) presented gain of an extra signal of *RUNX1* by FISH test, which was further found to be located on the abnormal chromosome 3 by map-back FISH in two cases (cases #105 and #106) (Fig. 4), most likely implying a potential *MECOM/RUNX1* fusion.

aCGH test results

Array CGH as an add-on test according to the clinician's request was performed in 4 cases (cases #40, #48, #108 and #123) with high blast counts and a complex karyotype. All cases exhibited complex array results with copy number variations (CNVs) involving multiple chromosomes and/or chromosomal segments, which were generally correlated with their karyotype analysis findings (Table 2**)**. For example, aCGH on case # 48 showed multiple CNVs involving chromosome 3, which were concordant with the atypical structural abnormalities of chromosome 3 detected by karyotype and the 3′*MECOM* deletion detected by interphase FISH analyses (Fig. 5). For case #108, a CNV of approximate 150 kb at 3q36.2 detected by aCGH, was too small to be detected by karyotype and FISH. In general, the *MECOM* rearrangement involved a balanced structural changes in most cases in this study, either through inversion, insertion or translocation, could not be detected by aCGH.

Discussion

Compared with previously reported 3q26.2 abnormalities summarized by De Braekeleer et al. [28], several *MECOM* rearrangement-related chromosomal abnormalities are identified for the first time in this study, e.g., the inv(3)(q26.2q28) (case #46, Fig. 6), t(3;10;21)(q26.2;p14;q22) (case #86) and ins(1;3)(p22;q24q26.2) (case #117). Case #118 with a

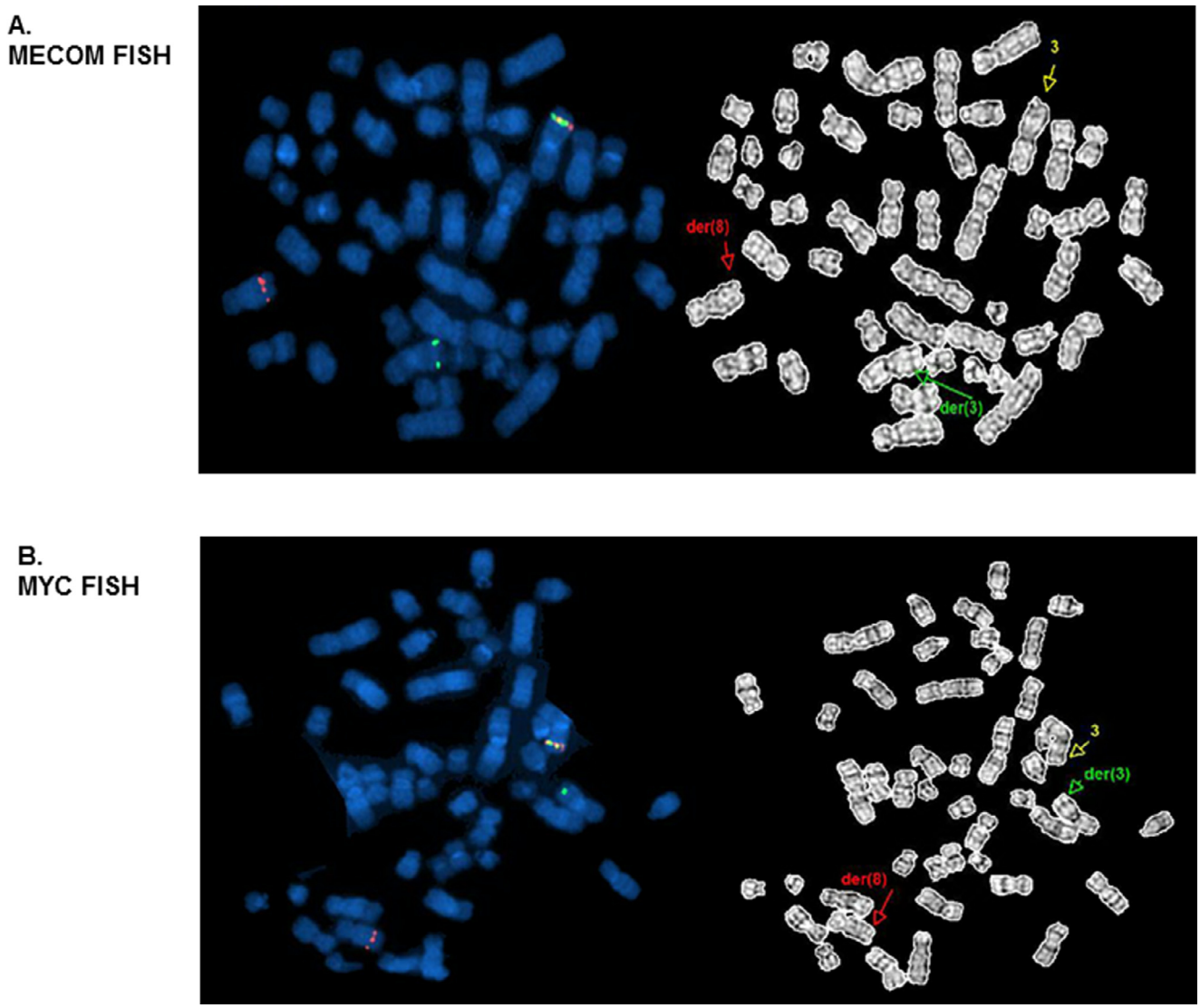

Fig. 3 A case of t(3;8) with simultaneous *MECOM* and *MYC* rearrangement (on the left side: the metaphase FISH images. On the right side: the inverted G-banded metaphase images to present the exact band levels of FISH signals. der(3): the abnormal chromosome 3; der(8): the abnormal chromosome 8). (A) MECOM FISH test indicates that the *5'MECOM* (red) signal is located on the abnormal chromosome 8; (B) MYC FISH test indicates that the *3'MYC* (green) signal is located on the abnormal chromosome 3.

5'MECOM signal on the abnormal chromosome 16, most likely an ins(16;3)(q?;q26.2q26.2) as described in the ISCN nomenclature (see [37]), and 6 other cases with a *MECOM* rearrangement but unknown underlying causative chromosomal abnormalities (cases #123-#128) are also first reported in this study. We also report here the first case of donor cell AML with *MECOM* rearrangement (case #54). The data presented are helpful to better understand the role of *MECOM* rearrangement (not only 3q26.2 aberrations as reported in literature) in myeloid malignancies and in the attribution of various mechanisms for a *MECOM* rearrangement derived from chromosomal abnormalities. As mentioned previously, FISH testing of *MECOM* was performed on the previously G-banded and karyotyped slides (map-back FISH) in more than 50% cases in this study. In combination with karyotype, both interphase and metaphase signal patterns and signal locations, this study has defined more precisely the structural abnormalities on chromosomes 3 as well as their partner chromosomes, especially in many cases with atypical chromosomal abnormalities.

The complexity of chromosomal aberrations related to *MECOM* rearrangement is high. In accord with many previous studies, the “classic” inv(3)(q21q26.2)/t(3;3)(q21;q26.2) represents 40–50% of all cases with *MECOM* rearrangement in myeloid neoplasms, 38% and 7%, respectively in this study [2,3,28,32,33]. However, a broad spectrum of “non-classic" chromosomal abnormalities involving 3q26.2 results in *MECOM* rearrangement in more than 50% of these cases (Table 1). There is an apparent synergy between two or more *MECOM* rearrangement related mechanisms as a combination of inversion, insertion, translocation and/or deletion was observed in a subset of cases in this study. We also detected simultaneous involvement of both copies of chromosome 3 in 10 cases (not including the “classic” cases),

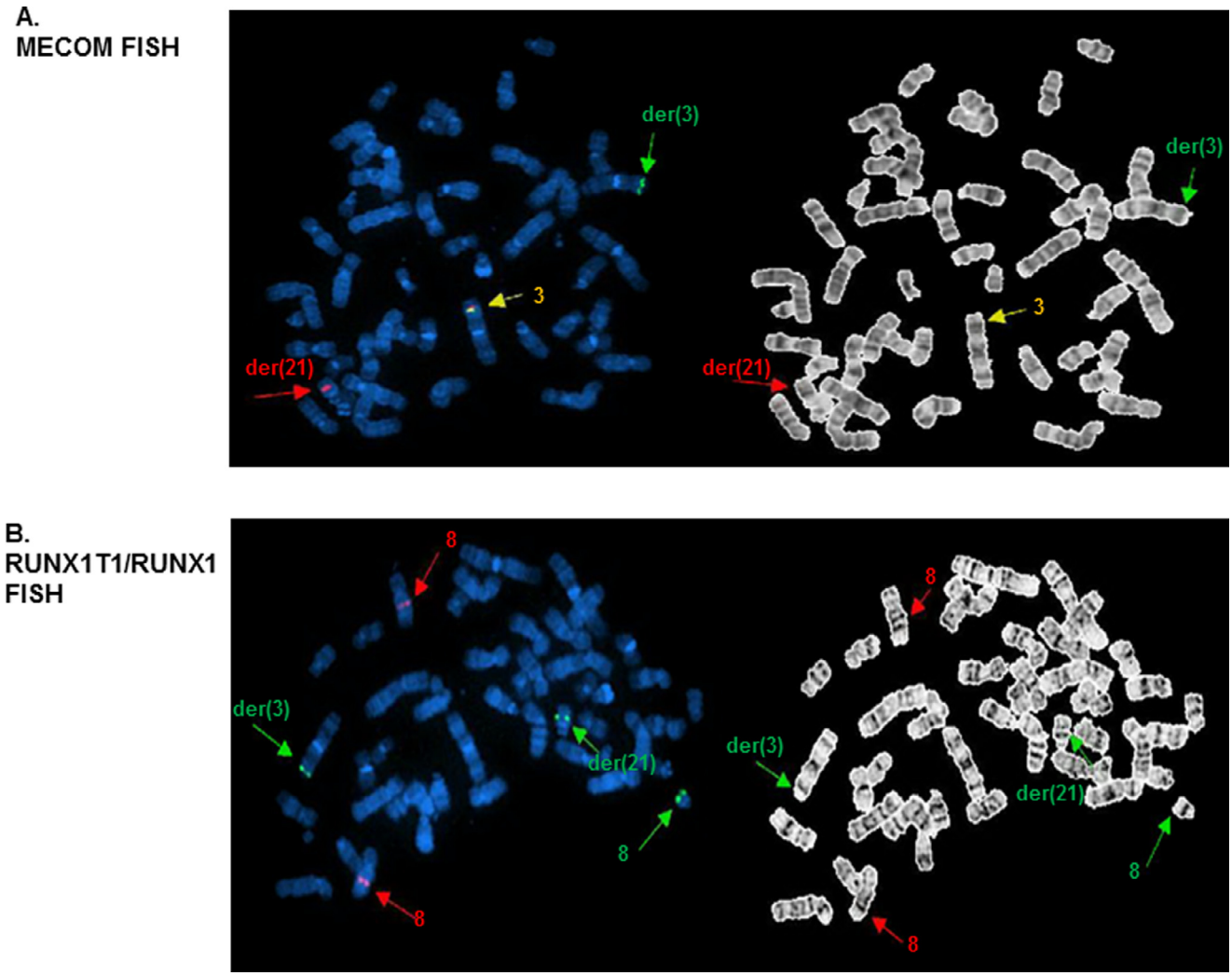

Fig. 4 A case of t(3;21) with simultaneous MECOM and RUNX1 rearrangement (on the left side: the metaphase FISH images. On the right side: the inverted G-banded metaphase images to present the exact band levels of FISH signals. der(3): the abnormal chromosome 3; der(21): the abnormal chromosome 21). (A) MECOM FISH test indicates that the *5'MECOM* (red) signal is located on the abnormal chromosome 21; (B) RUNX1T1/RUNX1 FISH test indicates that one extra copy of the *RUNX1* (green) signal is located on the abnormal chromosome 3, while *RUNX1T1* signals (red) are located on two normal chromosomes 8.

Table 2 The aCGH analysis results in 4 cases.

Case #	aCGH results
40	arr(GRCh38) 1p34.3(37379032_38487000) × 1,7p12.3p12.1(48356663_52874940) × 1
48	arr(GRCh38) 3p24.3(16466342_19927516) × 1,3q21.3q22.1(128622835_130279635)x1,3q26.1q26.2(166973261_168767303) × 1,5q11.2q35.3(52828952_180712263) × 1,7 × 1,12p13.32p12.3(5388552_18100420) × 1,12q14.3q15(67109153_70138199) × 1,12q23.3q24.32(108447499_126207097) × 1,17p13.3p11.2(76263_18316517) × 1
108	arr(GRCh38) 3q26.2(169166479_169325542) × 1,7q22.1q36.3(99594424_159118566) × 1
123	arr(GRCh38) 5q13.1q33.3(67646576_157317616) × 1,17p13.3p12(0_12238119) × 1,19q12q13.43(28793244_57243712) × 3,20q11.21q13.33(30690688_62477472) × 1

3 of them with rearrangement of both copies of *MECOM* gene [31].

As previously reported [18–20,24,27,31,35], *MECOM* rearrangement usually indicates dysregulation of MECOM expression and is associated with minimal to no response to chemotherapy and a poor clinical outcome in patients with myeloid malignancies, regardless the type of neoplasm or blast counts. Therefore, for all cases with 3q26.2 abnormality or any other chromosomal abnormalities with a suspicious *MECOM* rearrangement, the status of *MECOM* gene must be further confirmed by FISH or other methods. Five cases (cases #123, #124 and #126-#128) in this study without an

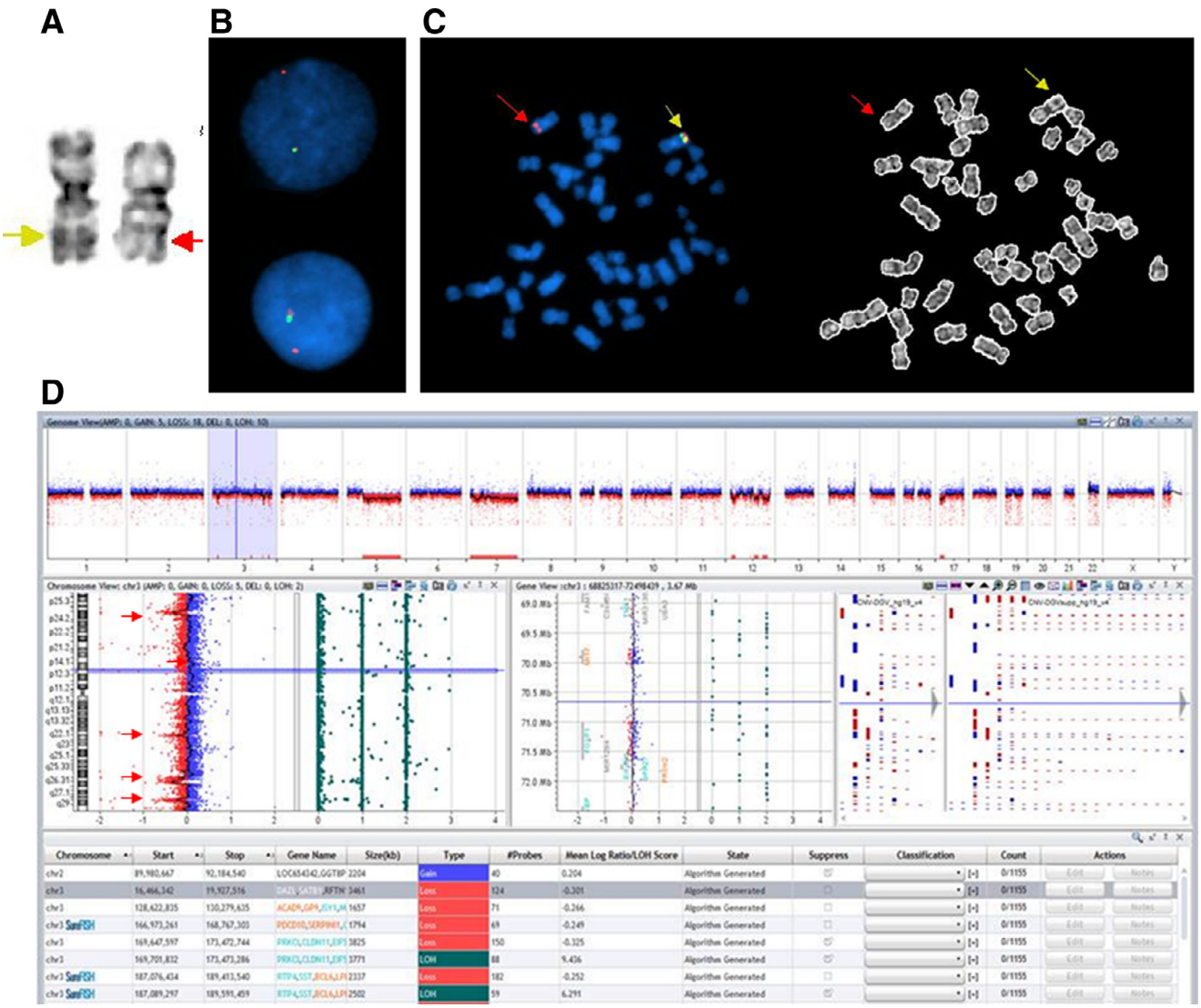

Fig. 5 Correlation of karyotype, MECOM FISH and aCGH analyses in Case #48. (A) Two copies of chromosomes 3 from one metaphase, left: normal chromosome 3 with intact *MECOM* signal (yellow arrow); right: abnormal chromosome 3 (described as der(3)del(3)(p25)inv(3)(q21q26.2)add(3)(q26.2)) with 5′*MECOM* signal (red arrow); both interphase (B) and map-back metaphase FISH (C) indicated a *MECOM* rearrangement with deletion of *3′MECOM*; (D) aCGH analysis presented CNVs involving multiple chromosomes, including several segments of the chromosome 3 (red arrow), which were consistent with the finding of a complex karyotype by conventional analysis in this case.

apparent 3q26.2 abnormality shown by conventional cytogenetic analysis were tested by FISH based on their past history of 3q26.2 abnormalities. In all 5 cases *MECOM* rearrangement was detected and 4 of them have died during a follow-up of 0–7 months. This phenomenon implies that the *MECOM* rearrangement in myeloid malignancies can be underestimated by conventional cytogenetic analysis. A deliberate prospective study of *MECOM* rearrangement by simultaneously conventional cytogenetic analysis and FISH testing is necessary, at least first in patients with a newly diagnosed myeloid neoplasm, especially for AML and MDS.

Different designs and coverages of MECOM FISH probe, such as dual-color breakapart MECOM probe, dual-color, fusion for *RPN1/MECOM, GATA2/MECOM, MECOM/RUNX1*, etc., are commercially available and have been applied in previous studies. Due to a wide variety of genomic breakpoint positions at 3q26.2 and unknown partner gene(s) for *MECOM* rearrangement in most cases, we have chosen a dual-color, MECOM BAP probe. This probe has worked very well, especially when a map-back FISH has been performed. It has overcome the low resolution of chromosomal analysis and insured a direct visualization of *MECOM* rearrangement on the abnormal chromosome(s) 3 and/or partner chromosome(s). We also observed in this study that approximately 20% of *MECOM* rearranged cases exhibited an “unbalanced” split signal pattern, mainly those cases with a t(2;3), t(3;21), insertions and/or unknown mechanism(s). The “unbalanced” split signal patterns have confused our technologists performing the interphase analyses, which had to be correlated with conventional cytogenetic and metaphase FISH analyses in

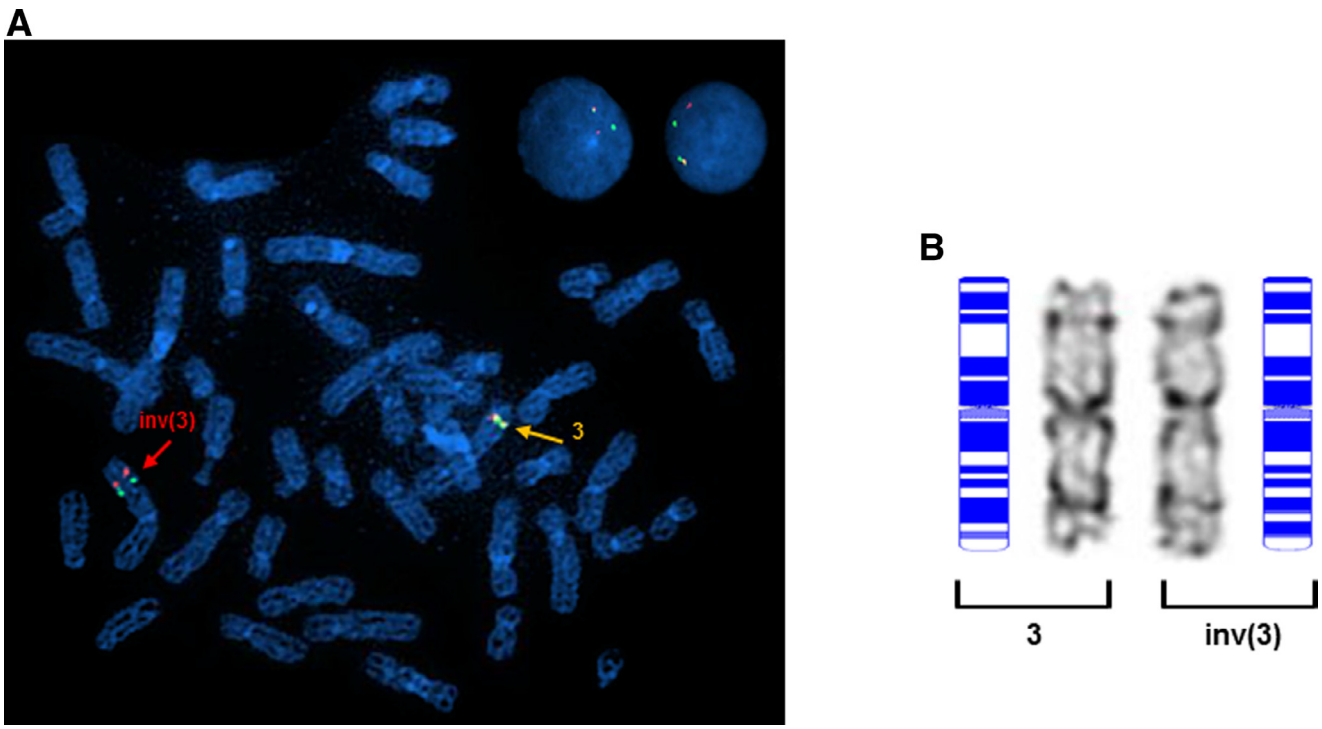

Fig. 6 An inv(3)(q26.2q28) with *MECOM* rearrangement (case #46). (A) Both metaphase and interphase FISH signal patterns indicate a *MECOM* rearrangement; (B) morphology of inv(3)(q26.2q28) and its representative ideogram (right) compared to the normal sister chromosome 3 and its representative ideogram (left) from the same metaphase cell shown in (A). The representative ideograms are drawn using an online software (http://www.cydas.org/index.html).

most cases. The "unbalanced" split signal pattern is believed to be caused by a breakpoint within the target of the *5′MECOM* probe. Therefore, a modification of the coverage of *5′MECOM* probe is necessary for these type of cases.

The frequencies of each type of *MECOM* rearrangement-related chromosomal aberration mentioned above are similar to those reported by Alpermann et al. [36] in a cohort of 116 *MECOM*-rearranged MDS and AML cases, in all of which rearrangement was confirmed by FISH. However, our results differ from the study of 252 patients by De Braekeleer et al. as well as a summary of 1038 cases published in the literature, where the frequencies from high to low are inv(3), t(3;3), t(3;21), t(3;12), t(2;3) and insertions respectively [28,32,33]. These discrepancies are most likely attributed to the following reasons: 1) most cases reported in these studies were based on conventional chromosomal analysis only; and 2) 13–14% of cases with 3q26.2 involvement in earlier studies were not further characterized for a specific type of chromosomal aberration (e.g., inversion, translocation, and insertion).

Some cases in this study with t(3;8), t(3;12) or t(3;21) have been confirmed for being positive for *MYC, ETV6* or *RUNX1* rearrangement simultaneously. These findings imply that likely a *MECOM/MYC, MECOM/ETV6* or *MECOM/RUNX1* fusion exists in these cases respectively. A further confirmation with *MECOM/MYC, MECOM/ETV6* or *MECOM/RUNX1* fusion probe have not been performed in these cases, since our map-back FISH approaches have clearly demonstrated the co-localization of both *MECOM* and the potential partner gene on the same affected chromosome(s) and at the same band level(s) (Figs. 3 and 4). All these fusion FISH probe can be necessary for cases like these in the future. According to our previous studies, these cases with potential fusion gene(s), such as *MECOM/MYC, MECOM/ETV6* or *MECOM/RUNX1* exhibited disease progression and prognosis similar or compatible to cases with inv(3)/*GATA2-MECOM* [18–20,22,24,27].

In summary, we present the largest study available on *MECOM* rearrangement in myeloid malignancies. A wide spectrum of chromosomal aberrations are associated with *MECOM* rearrangement, including but not limited to insertion, inversion and/or translocation of affected chromosome(s) 3 and many partner chromosomes. A confirmation of *MECOM* rearrangement by FISH testing, including map-back FISH approach in many cases, is necessary. A modification of coverage of current FISH probes may reduce the technical burden for certain cases.

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Conflict of interest

All authors declare that there is no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cancergen.2019.03.002.

References

[1] Arber DA, Brunning RD, Le Beau MM, Falini B, JW V. Acute myeloid leukemia with recurrent genetic abnormalities. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Arber DA, Hasserjian RP, Le Beau MM, Orazi A, Siebert R, editors. WHO classification of tumors of hematopoietic and lymphoid tissues. Lyon, France: International Agency for Research on Cancer; 2016. p. 130–49.

[2] Lugthart S, Groschel S, Beverloo HB, Kayser S, Valk PJ, van Zelderen-Bhola SL, Jan Ossenkoppele G, Vellenga E, van den Berg-de Ruiter E, Schanz U, Verhoef G, Vandenberghe P, Ferrant A, Kohne CH, Pfreundschuh M, Horst HA, Koller E, von Lilienfeld-Toal M, Bentz M, Ganser A, Schlegelberger B, Jotterand M, Krauter J, Pabst T, Theobald M, Schlenk RF, Delwel R, Dohner K, Lowenberg B, Dohner H. Clinical, molecular, and prognostic significance of WHO type inv(3)(q21q26.2)/t(3;3)(q21;q26.2) and various other 3q abnormalities in acute myeloid leukemia. J Clin Oncol 2010;28:3890–8.

[3] Groschel S, Lugthart S, Schlenk RF, Valk PJ, Eiwen K, Goudswaard C, van Putten WJ, Kayser S, Verdonck LF, Lubbert M, Ossenkoppele GJ, Germing U, Schmidt-Wolf I, Schlegelberger B, Krauter J, Ganser A, Dohner H, Lowenberg B, Dohner K, Delwel R. High EVI1 expression predicts outcome in younger adult patients with acute myeloid leukemia and is associated with distinct cytogenetic abnormalities. J Clin Oncol 2010;28:2101–7.

[4] Lugthart S, van Drunen E, van Norden Y, van Hoven A, Erpelinck CA, Valk PJ, Beverloo HB, Lowenberg B, Delwel R. High EVI1 levels predict adverse outcome in acute myeloid leukemia: prevalence of EVI1 overexpression and chromosome 3q26 abnormalities underestimated. Blood 2008;111:4329–37.

[5] Sun J, Konoplev SN, Wang X, Cui W, Chen SS, Medeiros LJ, Lin P. De novo acute myeloid leukemia with inv(3)(q21q26.2) or t(3;3)(q21;q26.2): a clinicopathologic and cytogenetic study of an entity recently added to the WHO classification. Mod Pathol Off J United States Canad Acad Pathol Inc 2011;24:384–9.

[6] Groschel S, Sanders MA, Hoogenboezem R, de Wit E, Bouwman BAM, Erpelinck C, van der Velden VHJ, Havermans M, Avellino R, van Lom K, Rombouts EJ, van Duin M, Dohner K, Beverloo HB, Bradner JE, Dohner H, Lowenberg B, Valk PJM, Bindels EMJ, de Laat W, Delwel R. A single oncogenic enhancer rearrangement causes concomitant EVI1 and GATA2 deregulation in leukemia. Cell 2014;157:369–81.

[7] Steinmetz B, Hackl H, Slabakova E, Schwarzinger I, Smejova M, Spittler A, Arbesu I, Shehata M, Soucek K, Wieser R. The oncogene EVI1 enhances transcriptional and biological responses of human myeloid cells to all-trans retinoic acid. Cell Cycle 2014;13:2931–43.

[8] Dreyfus F, Bouscary D, Melle J, Ribrag V, Guesnu M, Varet B. Expression of the Evi-1 gene in myelodysplastic syndromes. Leukemia 1995;9:203–5.

[9] Ohyashiki K, Ohyashiki JH, Shimamoto T, Toyama K. Pattern of expression and their clinical implications of the GATA family, stem cell leukemia gene, and EVI1 in leukemia and myelodysplastic syndromes. Leuk Lymphoma 1996;23:431–6.

[10] Buonamici S, Li D, Chi Y, Zhao R, Wang X, Brace L, Ni H, Saunthararajah Y, Nucifora G. EVI1 induces myelodysplastic syndrome in mice. J Clin Invest 2004;114:713–19.

[11] Ohyashiki K, Ohyashiki JH, Fujieda H, Shimamoto T, Kawakubo K, Nakazawa S, Suzukawa K, Morishita K, Toyama K. EVI1 expression associated with a 3q26 anomaly in a leukemia cell line derived from the blast crisis of chronic myeloid leukemia. Leukemia 1994;8:2169–73.

[12] Asou H, Eguchi M, Suzukawa K, Morishita K, Tanaka K, Date M, Hamamoto K, Kamada N. Establishment of a myeloid leukaemia cell line (Kasumi-4) with t(9;22;11)(q34;q11;q13), inv(3)(q21q26) and the EVI1 gene activation from a patient with chronic myelogenous leukaemia in blast crisis. Br J Haematol 1996;93:68–74.

[13] Ogawa S, Kurokawa M, Tanaka T, Tanaka K, Hangaishi A, Mitani K, Kamada N, Yazaki Y, Hirai H. Increased Evi-1 expression is frequently observed in blastic crisis of chronic myelocytic leukemia. Leukemia 1996;10:788–94.

[14] Mitani K. Molecular mechanism of blastic crisis in chronic myelocytic leukemia. Leukemia 1997;11(Suppl 3):503–5.

[15] Balatzenko G, Guenova M, Stoimenov A, Jotov G, Toshkov S. Philadelphia chromosome-positive chronic myeloid leukemia with p190(BCR-ABL) rearrangement, overexpression of the EVI1 gene, and extreme thrombocytosis: a case report. Cancer Genet Cytogenet 2008;181:75–7.

[16] Daghistani M, Marin D, Khorashad JS, Wang L, May PC, Paliompeis C, Milojkovic D, De Melo VA, Gerrard G, Goldman JM, Apperley JF, Clark RE, Foroni L, Reid AG. EVI-1 oncogene expression predicts survival in chronic-phase CML patients resistant to imatinib treated with second-generation tyrosine kinase inhibitors. Blood 2010;116:6014–17.

[17] Sato T, Goyama S, Kataoka K, Nasu R, Tsuruta-Kishino T, Kagoya Y, Nukina A, Kumagai K, Kubota N, Nakagawa M, Arai S, Yoshimi A, Honda H, Kadowaki T, Kurokawa M. Evi1 defines leukemia-initiating capacity and tyrosine kinase inhibitor resistance in chronic myeloid leukemia. Oncogene 2014;33:5028–38.

[18] Wang W, Cortes JE, Tang G, Khoury JD, Wang S, Bueso-Ramos CE, DiGiuseppe JA, Chen Z, Kantarjian HM, Medeiros LJ, Hu S. Risk stratification of chromosomal abnormalities in chronic myelogenous leukemia in the era of tyrosine kinase inhibitor therapy. Blood 2016;127:2742–50.

[19] Hu Z, Medeiros LJ, Wang W, Chen Z, Tang G, Hodjat P, Yang S, Fang L, Li Y, Verstovsek S, Hu S. 3q26.2/EVI1 rearrangement is associated with poor prognosis in classical Philadelphia chromosome-negative myeloproliferative neoplasms. Mod Pathol Off J United States Canad Acad Pathol Inc 2017;30:940–51.

[20] Hu Z, Hu S, Ji C, Tang Z, Thakral B, Loghavi S, Medeiros LJ, Wang W. 3q26/EVI1 rearrangement in myelodysplastic/myeloproliferative neoplasms: an early event associated with a poor prognosis. Leuk Res 2018;65:25–8.

[21] Yamamoto K, Okamura A, Sanada Y, Yakushijin K, Matsuoka H, Minami H. Marked thrombocytosis and dysmegakaryopoiesis in acute myeloid leukemia with t(2;3)(p22;q26.2) and EVI1 rearrangement. Ann Hematol 2013;92:1713–15.

[22] Lennon PA, Abruzzo LV, Medeiros LJ, Cromwell C, Zhang X, Yin CC, Kornblau SM, Konopieva M, Lin P. Aberrant EVI1 expression in acute myeloid leukemias associated with the t(3;8)(q26;q24). Cancer Genet Cytogenet 2007;177:37–42.

[23] Kubota Y, Ichinohe T, Yoshimura M, Itamura H, Hisatomi T, Fukushima N, Sueoka E, Kimura S. Acute myeloid leukemia with t(3;8)(q26;q24) complicated by diabetes insipidus. Ann Hematol 2016;95:653–5.

[24] Tang G, Hu S, Wang SA, Xie W, Lin P, Xu J, Toruner G, Zhao M, Gu J, Doty M, Li S, Medeiros LJ, Tang Z. t(3;8)(q26.2;q24) often leads to MECOM /MYC rearrangement and is commonly associated with therapy-related myeloid neoplasms and/or disease progression. J Mol Diagn 2018 Accepted.

[25] Iwase S, Furukawa Y, Horiguchi-Yamada J, Nemoto T, Takahara S, Kawano T, Sekikawa T, Ito K, Yamazaki Y, Kikuchi J, Morishita K, Yamada H. A novel variant of acute myelomonocytic leukemia carrying t(3;12)(q26;p13) with characteristics of 3q21q26 syndrome. Int J Hematol 1998;67:361–8.

[26] Bacher U, Schnittger S, Kern W, Trenn G, Weisser M, Haferlach T, Schoch C. Acute myeloid leukemia (AML) with t(8;21)(q22;q22) relapsing as AML with t(3;21)(q26;q22). Cancer Genet Cytogenet 2006;168:172–4.

[27] Yin CC, Cortes J, Barkoh B, Hayes K, Kantarjian H, Jones D. t(3;21)(q26;q22) in myeloid leukemia: an aggressive syndrome of blast transformation associated with hydroxyurea or antimetabolite therapy. Cancer 2006;106:1730–8.

[28] De Braekeleer M, Le Bris MJ, De Braekeleer E, Basinko A, Morel F, Douet-Guilbert N. 3q26/EVI1 rearrangements in myeloid hemopathies: a cytogenetic review. Future Oncol 2015;11:1675–86.

[29] Tang Z, Li Y, Wang W, Yin CC, Tang G, Aung PP, Hu S, Lu X, Toruner GA, Medeiros LJ, Khoury JD. Genomic aberrations involving 12p/ETV6 are highly prevalent in blastic plasmacytoid dendritic cell neoplasms and might represent early clonal events. Leuk Res 2018;73:86–94.

[30] Tang Z, Tang G, Wang SA, Lu X, Young KH, Bueso-Ramos CE, Alvarado Y, Medeiros LJ, Khoury JD. Simultaneous deletion of 3′ETV6 and 5′EWSR1 genes in blastic plasmacytoid dendritic cell neoplasm: case report and literature review. Mol Cytogenet 2016;9:23.

[31] Gu J, Patel KP, Bai B, Liu CH, Tang G, Kantarjian HM, Tang Z, Abraham R, Luthra R, Medeiros LJ, Lin P, Lu X. Double inv(3)(q21q26.2) in acute myeloid leukemia is resulted from an acquired copy neutral loss of heterozygosity of chromosome 3q and associated with disease progression. Mol Cytogenet 2015;8:68.

[32] De Braekeleer M, Gueganic N, Tous C, Le Bris MJ, Basinko A, Morel F, Douet-Guilbert N. Breakpoint heterogeneity in (2;3)(p15-23;q26) translocations involving EVI1 in myeloid hemopathies. Blood Cells Mol Dis 2015;54:160–3.

[33] De Braekeleer E, Douet-Guilbert N, Basinko A, Bovo C, Gueganic N, Le Bris MJ, Morel F, De Braekeleer M. Conventional cytogenetics and breakpoint distribution by fluorescent in situ hybridization in patients with malignant hemopathies associated with inv(3)(q21;q26) and t(3;3)(q21;q26). Anticancer Res 2011;31:3441–8.

[34] Haferlach C, Bacher U, Grossmann V, Schindela S, Zenger M, Kohlmann A, Kern W, Haferlach T, Schnittger S. Three novel cytogenetically cryptic EVI1 rearrangements associated with increased EVI1 expression and poor prognosis identified in 27 acute myeloid leukemia cases. Genes Chromosomes Cancer 2012;51:1079–85.

[35] Lin P, Lennon PA, Yin CC, Abruzzo LV. Chronic myeloid leukemia in blast phase associated with t(3;8)(q26;q24). Cancer Genet Cytogenet 2009;193:119–22.

[36] Alpermann T, Haferlach C, Fasan A, Schindela S, Kern W, Haferlach T. Prognosis of Mecom(EVI1)-rearranged MDS and AML patients strongly depends on accompanying molecular mutations but not on blast counts. Blood 2015;126:1372.

[37] Tang Z, Tang G, Hu S, Patel KP, Yin CC, Wang W, Lin P, Toruner GA, Ok CY, Gu J, Lu X, Khoury JD, Medeiros LJ. Data on MECOM rearrangement-driven chromosomal aberrations in myeloid malignancies. Data Brief 2019 DIB-D-19-00629, submitted for publication.