Brief Report

MYELOID NEOPLASIA

Utility of peripheral blood for cytogenetic and mutation analysis in myelodysplastic syndrome

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Key Points

- There is 100% concordance in the cytogenetic and mutation profile between PB and BM in myelodysplastic syndrome.

Recent studies have shown that more than 80% of bone marrow (BM) samples from patients with myelodysplastic syndrome (MDS) harbor somatic mutations and/or genomic aberrations, which are of diagnostic and prognostic importance. We investigated the potential use of peripheral blood (PB) and serum to identify and monitor BM-derived genetic markers using high-resolution single nucleotide polymorphism array (SNP-A) karyotyping and parallel sequencing of 22 genes frequently mutated in MDS. This pilot study showed a 100% SNP-A karyotype concordance and a 97% mutation concordance between the BM and PB. In contrast, mutation analysis using Sanger sequencing of PB and serum-derived DNA showed only 65% and 42% concordance to BM, respectively. Our results show the potential utility of PB as a surrogate for BM for MDS patients, thus avoiding the need for repeated BM aspirates particularly in elderly patients and those with fibrotic or hypocellular marrows. (*Blood*. 2013;122(4):567-570)

Introduction

The myelodysplastic syndromes (MDSs) are clonal disorders of hematopoiesis that occur predominantly in the elderly (median age 72 years) and are characterized by morphologic dysplasia, ineffective hematopoiesis, peripheral blood (PB) cytopenias, chromosomal aberrations, and propensity to myeloid leukemic transformation. The advent of high-throughput and high-resolution techniques for genetic analysis has shown that more than 80% of MDS patients harbor somatic mutations and/or genomic aberrations in their bone marrow (BM), which provide pathogenetic as well as diagnostic and prognostic insights into this disease.[1-4] Frequent BM aspirates may be required for morphological[5] and genetic assessment, especially after BM transplant. In addition, in a significant patient proportion, the BM is hypocellular (10% to 15%)[6] and/or fibrotic (17%),[7] making the aspiration procedure painful and uncomfortable, especially in the elderly. In MDS the molecular analysis of copy number changes and genetic mutations has been done primarily on BM-derived DNA samples. Previous studies that used fluorescent in situ hybridization (FISH) and single nucleotide polymorphism arrays (SNP-As) to compare the karyotype concordance between BM and PB showed the usefulness of this approach. However, a comprehensive genetic analysis to compare the karyotype and mutation profile between BM and PB in MDS has not been performed.[8-11]

In this pilot study we investigated the presence of BM-derived genetic markers in both PB and serum from the same patients using high-resolution SNP-A karyotyping, 454 parallel sequencing (454-PS), and Sanger sequencing of 22 genes most frequently mutated in MDS and acute myeloid leukemia (AML).

Study design

Genomic DNA from PB and BM was extracted (Qiagen) from frozen cell pellets and 100 ng was whole genome amplified (WGA; Qiagen), both per manufacturer's protocols. Serum DNA was purified from 200 μL of serum using a modified sodium iodide/Triton-based lysis followed by isopropanol precipitation as described.[12] Affymetrix SNP 6.0 array (SNP-A) karyotyping and 454-PS of all exons of *DNMT3a*, *RUNX1*, *CEBPα*, *TP53, EZH2,* and *ZRSR2* and mutation "hot spots" for *NPM1, FLT3*, *ASXL1*, *IDH1*, *IDH2*, *MPL*, *JAK2*, *BRAF*, *cCBL*, *NRAS*, *KRAS,* C-*KIT, SF3B1, SRSF2,* and *U2AF35* were performed and analyzed as previously described.[13,14] *TET2* was analyzed using Sanger sequencing. Independent validation for all mutations was performed using Sanger sequencing of unamplified genomic DNA. Polymerase chain reaction (PCR) conditions for serum were identical to those for PB; however, a second 10-cycle PCR reaction using nested primers (US1–GTAGTGCGATGGCCAGT, US2–CAGTGTGCAGCGATGAC) was required to provide adequate amplicon yield for Sanger sequencing. The study was approved by the local research ethics committee under project 0033 and conducted in accordance with the Declaration of Helsinki.

Results and discussion

Karyotype analysis

Karyotype aberrations were assessed using SNP-A on PB samples from 31 MDS patients, from whom metaphase cytogenetics (MC) and BM SNP-A karyotypes were available. These consisted of the

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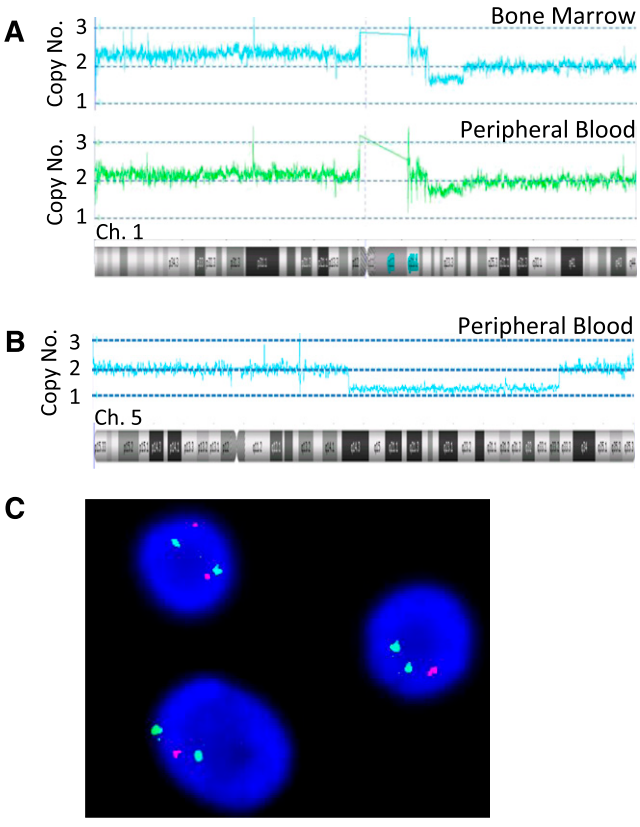

Figure 1. SNP array copy number ideograms of chromosome 1 (USN1902) and chromosome 5 (USN9124). (A) Comparison of chromosomal abnormalities present in BM and PB. Copy number variations present in the BM are clearly visible in PB; however, they are slightly reduced (BM gain CN-2.3, loss CN-1.7; PB gain CN-2.1, loss CN-1.85). (B) Representative ideogram of chromosome 5 from a patient with 5q syndrome showing del(5q). (C) FISH analysis showing 2 cells with del5q and 1 with a normal chromosome 5 from the PB of a 5q-syndrome patient. The red and the green probes are the 5q31 EGR1 and the 5p15 D15523/D55721 probes, respectively.

following: normal karyotype (n = 9), del5q (n = 9), del7q/-7 (n = 5), trisomy 8 (n = 2), complex (n = 4), isodiXq13 (n = 1), and t(2:4)(q33;q27) (n = 1).

Concurrent BM and PB samples were available for 22 patients; nonconcurrent PB samples, median of 364 (14 to 1379) days after initial BM aspirate, were available for the remaining 9 patients. SNP-A karyotyping showed 100% concordance between BM and PB karyotype, except for 1 sample from a patient (USN1902) with a complex karyotype comprising 30 chromosomal aberrations in the BM, of which only 15 could be detected in the PB, suggesting the presence of multiple mosaic clones (Figure 1A; supplemental Table 1, available on the *Blood* web site).[15,16] Comparison of the PB SNP-A karyotypes with BM metaphase cytogenetics showed 100% concordance, except for the presence of 9 identical regions of uniparental disomy (UPD) in the PB and BM of 7 cases (UPD18q12.1-q21.31, UPD14q11.2-q32.33, UPD3p14.1-p13, UPD3q25.1-q26.31, UPDXq21.33-q23, UPD1p36.33-p13.3, UPD13q11-q34, UPD11p15.5-p13, and UPD15q15.1-q26.3). Translocations identified in 5 cases [t(7:21), der(1:7) (n = 2), t(1;6;8)(^{32}P;p21;q12), and t(2;4)(q33;q27)] by MC in the BM were not detected using SNP-A analysis either in the PB or BM (supplemental Table 1). Of note, in 8 cases with an isolated 5q deletion in the BM as assessed by MC, the 5q deletion could also be detected in the PB using SNP-A as well as FISH analysis (Figure 1B-C; supplemental Table 1).

Mononuclear cells from PB in general showed a lower clone size in comparison with those of BM, as indicated by the smaller mean copy number (CN) for chromosomal aberrations (PB vs BM): deletions (CN of 1.8 vs 1.6) and gains (CN of 2.2 vs 2.4; Figure 1A).

Mutational analysis

Mutational analysis of BM-derived genomic DNA from 21 MDS patients showed 38 mutations comprising *TET2, SF3B1*, and *TP53* (n = 7 cases each); *ASXL1* (n = 3); *DNMT3a* and *FLT3* (n = 3 cases each); *U2AF35* (n = 2); and *NRAS,* c*CBL*, *JAK2, IDH2,* and *SRSF2* (n = 1 case each). Concurrent PB was available for 13 patients and nonconcurrent PB samples were available for the other 8 patients (median 409 [96 to 1073 days]). Mutational analysis of concurrent and nonconcurrent PB genomic DNA showed that 37 of the 38 (97%) mutations present in BM could also be detected in PB, with the exception of 1 NRAS mutation seen in a relatively small BM clone (size, 11%) that was undetectable in a concurrent PB sample (USN1533; Table 1). Interestingly, USN2233 (PB) was analyzed post azacitidine treatment and showed a mutation in TP53 (V157F, 1.5%) that was undetectable using Sanger sequencing, which was consistent with a normal PB SNP-A karyotype also observed in the respective BM (USN2232). This mutation was detected in the presentation sample (USN1894) at a clone size of 24% with concomitant genomic aberrations: on del5p15.33-p14.3, del5q11.2-q33.3, del17p13.3-p11.2, and gain19q12-q13.43 (supplemental Figure 1).

Similar to SNP-A karyotype analysis, the mutant clone size in PB was lower (median 25% [1.5% to 50%]) but was not significantly different (P = .4) from the BM clone size (median 33% [5% to 68%]), which is in agreement with previous karyotype studies in PB.[10] In contrast to the results obtained using 454-PS that showed 97% concordance, Sanger sequencing resulted in only 65% concordance (smallest detected clone size was 21%) between BM and PB. These differences are attributable to the superior sensitivity of parallel sequencing technology to identify low-level clones. In addition, we compared the mutation profiles of unamplified and WGA PB DNA from 2 patients with mutations in *TET2* and *U2AF35* (Table 1). Contrary to a recent report, this analysis showed similar mutation profiles for both DNA samples.[12]

To further study the utility of these procedures, we isolated DNA from serum of 12 patients with a total of 19 mutations in their BM. Analysis using electrophoresis and DNAOK! reagent (Web Scientific) showed highly fragmented serum-derived DNA unsuitable for SNP-A analysis. Sanger sequencing correctly identified only 8 mutations with no preference for any of the genes (42% concordance); 6 mutations were identified as wild type and 5 samples failed to amplify (Table 1).

In conclusion, our study showed excellent concordance both for SNP-A karyotype and mutational analyses between BM and PB, albeit with a lower clonal burden in PB, using FISH, SNP-A, and 454-PS that was independent of the differential PB profile of the patient cohort (supplemental Table 2). Patient serum is not recommended for mutation detection. The detection of regions of UPD in both PB and BM provides additional prognostic information because UPD on chromosome 7q has been associated with more aggressive clinical behavior in MDS and UPD on 5q may identify patients with potential response to lenalidomide.[3,17-19] Our results show the feasibility of using PB for initial genetic diagnostic screening of patients with MDS and sequential monitoring of

Table 1. Mutations detected in BM, PB, and serum using 454-PS and Sanger sequencing

PB USN	WHO Classification	Gene	Mutation Site	PB Clone Size (%)	PB Sanger	BM USN	BM Clone Size (%)	Serum Sanger
*682	RAEB-1	TET2	D1844G	S	+	683	68	—
		U2AF35	S34F	9	—		9	—
*1196	RARS-T	JAK2	V617F	21	+	1190	24	
		SF3B1	K700E	25	+		41	
*1334	RAEB-2	DNMT3A	F751V	9	—	1280	25	
			P904S	10	—		20	
*1337	sAML	FLT3	ITD	S	+	1284	40	+
		CBL	Y368S	S	+		8	—
*1533	RAEB-2	ASXL1	G646WfsX12	8	—	1532	22	
		NRAS	Q61R	Fail	—		11	
		FLT3	ITD	2	—		5	
		SRSF2	P95H	50	+		45	
*1591	RAEB-1	TP53	L43X	16	—	1590	40	Fail
		TP53	C238Y	15	—		41	Fail
		TET2	R1878H	S	+		6	Fail
*1599	RCMD	U2AF35	Q157P	17	—	1600	27	
1606	RCMD	DNMT3A	W313X	44	+	955	32	
*1637	RCMD	SF3B1	E622D	S	+	1636	40	+
*1682	RCMD	TET2	I1195V	S	+	1681	47	
*1809	RAEB-1	TET2	L1322fs	S	+	1808	22	+
1850	tMDS	TP53	Y220C	30	+	1393	42	+
		TP53	Q331H	23	+		41	Fail
*1902	RAEB-1	TP53	G266E	15	—	1898	28	
		TET2	Y1148C	S	+		25	
		TP53	G325fsX12	16	—		28	
2233	RAEB-2	TP53	V157F	1.5	—	1894	24	Fail
2301	RCMD	ASXL1	R860X	31	+	2025	34	+
*2734	RCMD	SF3B1	R625C	45	+	2733	46	
		FLT3	ITD	50, 10	+		80	
		TET2	S5835X	S	+		35	
*7664	RARS	TET2	Q652X	S	+	7660	34	+
		SF3B1	K700E	18	—		30	+
7781	RAEB-2	ASXL1	Q708X	40	+	4242	35	+
8748	RCMD	IDH2	R140Q	4.5	—	4323	35	—
		SF3B1	K700E	1.6	—		40	—
9235	RCMD	DNMT3A	W581S	25	+	5087	22	
		SF3B1	H662Q	42	+		30	
9999	RARS	SF3B1	H662Q	40	+	4370	42	—

TET2 was analyzed using 454-PS in BM and Sanger sequencing in both BM and PB. Serum was analyzed using Sanger sequencing.

+/− mutation detectable or undetectable using Sanger sequencing; fail, uninformative result; S, Sanger sequencing of TET2; USN, unique sample number; USN, patients who have been analyzed using 454-PS and Sanger sequencing from unamplified and whole genome amplified DNA; USN*, patients with concurrent BM and PB samples; WHO, World Health Organization.

disease clones following treatment, thus forming a prelude for validation in larger studies.

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Authorship

Contribution: G.J.M. conceived the study; A.M.M., H.A., A.K., S.S., S.M., F.M., A.E.S., and J.G. performed experiments; A.M.M., G.J.M., and J.G. contributed to design, analysis, and manuscript preparation; and A.K. and G.J.M. provided clinical details and analysis.

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