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.

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 morphological5 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 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)

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, CEBPa, 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–CAGTGTCACGGATGAC) 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


J.G. and G.J.M. contributed equally to this study.

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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 NRCAS, cCBL, 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. 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.

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 Scientif) showed highly fragmented serum-derived DNA unsuitable for SNP-A analysis. Sanger sequencing correctly identified 2 mutations in serum DNA, and no mutations were detected in serum-derived DNA when using the DNAOK! reagent (Web Scientific).

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

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Authorship


Conflict-of-interest disclosure: The authors declare no competing financial interests.

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