Array comparative genomic hybridization of peripheral blood granulocytes of patients with myelodysplastic syndrome detects karyotypic abnormalities

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Introduction

The myelodysplastic syndromes (MDS) are a group of clonal stem cell disorders characterized by ineffective hematopoiesis with abnormal differentiation and maturation of myeloid cells, resulting in one or more peripheral cytopenias.\(^1,2\) MDS is thought to be the most common myeloid malignancy, with an overall incidence of about 3-5/100,000 individuals per year increasing to about approximately 100/100,000 per year in individuals over 70 years of age.\(^3,4\) MDS likely occurs due to genetic changes in a primitive CD34\(^+\) hematopoietic cell that gives rise to abnormal progeny cells.\(^5-9\) Although genetic abnormalities are thought to be present in all MDS patients, standard cytogenetics on bone marrow samples only detects an abnormal karyotype in approximately 50% of low-risk MDS patients.\(^1,2,8\) We and others recently identified cryptic copy number alterations in bone marrow cells of a large proportion (18-68%) of patients with low-risk MDS and a normal karyotype, using high-resolution array platforms, suggesting that structural genomic abnormalities are more prevalent than expected.\(^9-12\) In addition, we showed that the presence of these alterations has prognostic value in low-risk MDS.\(^9\) These findings suggest that the use of array-based platforms, such as array comparative genomic hybridization (aCGH) or single-nucleotide polymorphism arrays on bone marrow cells, are useful tools in the diagnosis and prognosis of patients with MDS.

Cytogenetic studies are not always performed or successful in MDS. A recent report showed that only approximately 60% of MDS patients undergo a bone marrow evaluation.\(^4\) Therefore, we investigated whether peripheral blood granulocytes can be used as an alternative source of cells for obtaining cytogenetic information using genomic arrays. We utilized high-resolution bacterial artificial chromosome (BAC) aCGH analysis to compare the cytogenetic alterations in matched CD34\(^+\) bone marrow-derived cells and peripheral blood granulocytes from MDS patients.
Study Design

Sample Collection
Bone marrow and peripheral blood samples were obtained from 11 MDS patients (MDS, n = 8 and MDS/MPD, n = 3) at diagnosis following informed consent (Table 1; age range from 60 to 85 years, with a median of 66 years and a mean of 70 years). All protocols were approved by the Clinical Research Ethics Board of the British Columbia Cancer Agency/University of British Columbia.

Cell isolation
CD34+ cells were positively selected from cryopreserved marrow by immunomagnetic separation according to the manufacturer’s instructions (Stem Cell Technologies, Vancouver, BC) with a final purity of >70%. Peripheral blood granulocytes were isolated using Ficoll-Hypaque density-gradient centrifugation according to the manufacturer’s instructions (Stem Cell Technologies, Vancouver, BC).

Whole genome tiling-path aCGH analysis
Details of whole genome array CGH including DNA extraction, labeling and hybridization as well as image analysis have been described previously.9,13,14 This platform has a theoretical resolution of ~50 kb.13 SeeGH custom software was used to visualize all data as log ratio plots.14,15 Clones with SD values between duplicate spots of more than 0.1 were filtered from the raw data. A region was considered altered when a minimum of 2 overlapping consecutive clones showed the change. A hidden Markov model (HMM) algorithm was used to verify breakpoints of genomic alterations identified by visual inspection as described.16

Statistical Analysis
Differences in genomic alterations between CD34+ cells and granulocytes of MDS patients were analyzed using the Mann-Whitney test. Analysis was carried out using GraphPad Prism4 (GraphPad Software, San Diego, CA).
Results and Discussion

High-resolution array CGH was performed on 11 DNA samples of matched CD34+ cells and granulocytes from MDS or MDS/MPD patients (Table 1S). Four of eleven patients had known cytogenetic abnormalities as identified by conventional karyotyping with good concordance between conventional cytogenetics and array CGH. Patient 2 had a deletion 11 from q14.1 to q24.3 detected in 15/15 metaphases, which was identified in both the CD34+ cells and the granulocytes by aCGH. Patient 5 had a deletion 5 from q23.1 to q31.2 detected in 11/20 metaphases and +8 in 4/20 metaphases by conventional cytogenetics. By aCGH we identified deletion 5 from q23.1 to q31.2 in the CD34+ cells and granulocytes, but could not detect the +8 in either CD34+ cells or granulocytes, consistent with an aCGH detection threshold of 25-30% abnormal cells.13 Additionally, in patient 5 we detected a deletion 20 from q11.21 to 13.33, which was not clearly resolved by conventional karyotyping. Patient 9 had +8 in 14/14 metaphases and this abnormality was detected in both CD34+ cells and granulocytes by aCGH. Patient 10 had an isodicentric chromosome involving the short arm of chromosome X with a breakpoint at q13 in 18/25 metaphases, which was seen in both the CD34+ cells and granulocytes with aCGH.

Three patients (patient 4, 7 and 8) had a normal karyotype by conventional cytogenetics and array CGH did not reveal gross (>3 Mb) chromosomal alterations in the CD34+ cells or granulocytes. In four patients (patient 1, 3, 6 and 11) conventional karyotyping either failed or was not performed. One of these patients (patient 11) revealed a partial trisomy 9 from q33.3 to q34.3 as well as trisomies 19 and 22 by aCGH in both CD34+ cells and granulocytes, whereas the other three patients did not show any large chromosomal abnormalities. These findings are in agreement with previous studies showing that circulating granulocytes contain a detectable population that arises from the malignant clone in the majority of MDS patients.17,18

As the resolution of array CGH is significantly greater than that of conventional cytogenetics 19, we investigated whether cryptic alterations in CD34+ cells of MDS patients are also present in their peripheral blood granulocytes. We found no significant difference (P = 0.19) in total genomic alterations seen in CD34+ cells (mean total genomic alterations: 57.5 Mb ± 70.2) and granulocytes (mean total genomic alterations: 39.3 Mb ± 67.6) of 11 MDS patients using aCGH (Figure 1A). However, when genomic alterations which could potentially be detected by conventional karyotyping (>10 Mb) were excluded, a significantly higher level of cryptic
alterations was seen in CD34⁺ cells (mean total cryptic alterations 13.3 Mb ± 10.3, range 3.2 - 30.2 Mb) of MDS patients compared to granulocytes (mean total cryptic alterations 0.3 Mb ± 0.5, range 0.0 – 1.6 Mb) (P < 0.0003) (Figure 2A).

Previously we reported that cryptic changes of up to 2.6 Mb in total can be found in CD34⁺ cells of healthy elderly individuals.⁹ Therefore, we considered >3 Mb of cryptic changes to be disease related. None of the 11 MDS patients analyzed in this study had >3 Mb changes in their granulocytes excluding large karyotypic alterations (range 0.0 – 1.6 Mb). For example, Figure 2B shows two cryptic alterations seen in the CD34⁺ cells of MDS patient 6, and amplification 1 is clearly absent in granulocytes. Although we cannot exclude that amplification 2 is present in a proportion of granulocytes, it would not fulfil our criteria for a cryptic alteration (see Material and Methods). Therefore in the absence of data from the CD34⁺ cells, we would not have considered amplification 2 to be present in the granulocytes. This finding suggests that a large proportion of circulating granulocytes of MDS patients likely do not arise from the malignant clone, but rather represent residual normal hematopoiesis, in keeping with previous observations by fluorescence in situ hybridization (FISH) showing that even dysplastic circulating neutrophils may represent the normal population.¹⁹,²⁰

Our findings suggest that in patients with failed standard cytogenetics, aCGH studies on the peripheral blood may be useful, as large genomic alterations are usually detected in peripheral blood granulocytes. In particular, detection of del(5q) in circulating granulocytes may be a useful alternative to a marrow examination in patients being considered for lenalidomide therapy. Nevertheless, cryptic alterations present in CD34⁺ bone marrow cells of patients with MDS cannot be readily detected in peripheral blood granulocytes and are therefore not an optimal diagnostic substitute in MDS patients with a normal karyotype, even when using high-resolution genomic platforms.

References