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Reduction in multi-lineage and erythroid progenitors distinguishes myelodysplastic syndromes from non-malignant cytopenias

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ABSTRACT

We studied the diagnostic role of CFC assays in myelodysplastic syndromes (MDS) using CFC data from bone marrow (BM) and peripheral blood (PB) of 221 MDS patients, 51 patients with non-malignant causes of cytopenia and/or dysplasia and 50 normal controls. A consistent decrease in BM but not PB multi-lineage and erythroid progenitor frequencies was seen in patients with MDS compared to controls ($P < 0.05$). Automated distinction showed a sensitivity of $87 \pm 6\%$ and a specificity of $71 \pm 11\%$ in classifying MDS patients. In conclusion, a defect in early hematopoietic progenitor activity, in particular erythroid activity, distinguishes MDS from non-MDS.

1. Introduction

Myelodysplastic syndromes (MDS) are a heterogeneous group of clonal disorders arising from a primitive $CD34^+$ cell and characterized by peripheral cytopenias, dysplasia and ineffective hematopoiesis [1–4]. The incidence of MDS is likely underestimated but it is thought to be the most common hematopoietic malignancy with an incidence of about 10 cases per 100,000 people [5,6]. Classification systems for MDS are based on morphology or prognostic parameters. The French–American–British (FAB) classification defined five major subtypes and provided an initial morphology-based classification framework [7]. In 2002, the World Health Organization (WHO) described a refined version of the FAB system to which cytogenetic parameters were added and this system was revised further in 2008 [8]. The International Prognostic Scoring System (IPSS) is another classification system for prognostic stratification of newly diagnosed MDS patients that uses the blast count, cytogenetic data and number of peripheral cytopenias [9].

Cytogenetic abnormalities are found in approximately 50% of patients with primary MDS and in about 80% of patients with therapy-related MDS [2,9,10]. In patients with a normal karyotype, the diagnosis of MDS can be difficult as typical findings of the disease, i.e., cytopenias and dysplasia, often occur in other malignant and non-malignant conditions [11–15]. To address these issues, consensus statements from a recent working conference were published outlining minimal diagnostic criteria for MDS [16]. The working group proposed that hematopoietic progenitor assays could aid in the diagnosis of MDS if the colony-forming cells (CFCs) were markedly and persistently reduced [16]. However, details regarding the type of CFCs that were reduced, the level of reduction expected and the duration of the reduction that had diagnostic validity were not specified.

CFC assays assess the quantity and quality of erythroid and myeloid progenitors [17,18]. Abnormalities in the growth of MDS cells *in vitro* have been described, but most studies comprise relatively few cases (<25 patients) and all subtypes of MDS have usually not been represented [19–22]. Here we report the results of a large retrospective study designed to examine the bone marrow (BM) and peripheral blood (PB) CFC data accrued from 221 MDS patients and 51 patients with cytopenias secondary to systemic disease assays using the same standardized culture conditions. Comparison of the CFC data from different subtypes of MDS

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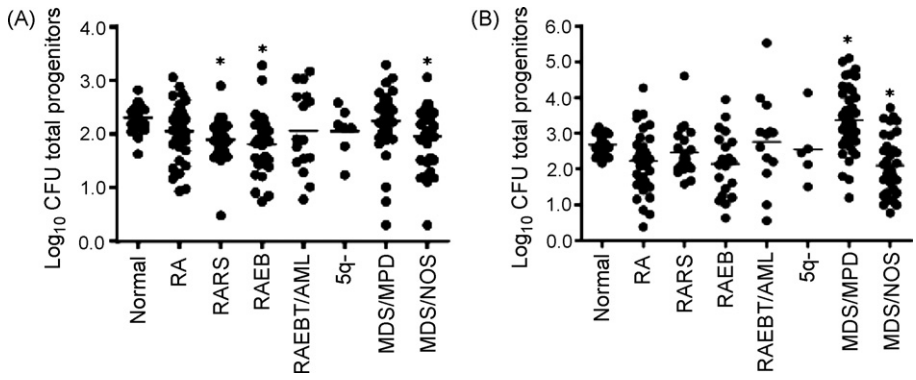

Fig. 1. Enumeration of $\log_{10}$ total CFCs detected in BM and PB samples from patients with MDS. (A) $\log_{10}$ total CFCs per 1×10^5 BM cells in normal controls ($n = 50$) and various MDS subtypes (RA, $n = 48$; RARS, $n = 24$; RAEB, $n = 28$; RAEB-T/AML, $n = 20$; 5q- syndrome, $n = 7$; MDS/MPD, $n = 43$; MDS-NOS, $n = 38$). (B) $\log_{10}$ total CFCs per ml PB in normal controls ($n = 30$) and the MDS subtypes (RA, $n = 41$; RARS, $n = 21$; RAEB, $n = 22$; RAEB-T/AML, $n = 17$; 5q- syndrome, $n = 5$; MDS/MPD, $n = 42$; MDS-NOS, $n = 37$). *$P < 0.05$.

and normal controls revealed some CFC data to have diagnostic value.

2. Materials and methods

2.1. Patients

Records of BM ($n = 208$) and PB ($n = 177$) CFC assays of 221 MDS patients performed between 2000 and 2005 in the Stem Cell Assay laboratory located at the Terry Fox Laboratory in Vancouver were reviewed with approval of the Research Ethics Board of the BC Cancer Agency and the University of British Columbia. CFC assays were performed as part of a routine work-up for MDS at diagnosis. Patients were assigned to particular subtypes according to the FAB classification with RAEB-T/AML used to group patients with a blast count of 20–30%. Patients with 5q- syndrome were separated and patients with chronic myelomonocytic leukemia (CMML) were classified as MDS/myeloproliferative syndrome (MPD). Patients who could not be classified based on morphology or cytogenetics were grouped as MDS-not-otherwise-specified (NOS). One patient with refractory anemia with ringed sideroblasts (RARS) was excluded as she may have received erythropoietin treatment at the time of the CFC assays. The white blood cell (WBC) count of 160/177 MDS patients, who had PB CFC assays done, was obtained. Patients with hematologic malignancies other than MDS were excluded from this study. MDS patients with normal karyotype, blasts <5% and an IPSS score $\leq$1.0 ($n = 68$) were compared to patients with cytopenias secondary to systemic disease (nutritional deficiencies, toxins and liver/renal disease) ($n = 51$). Results were compared to normal controls (BM, $n = 50$; PB, $n = 30$).

2.2. CFC assays

CFC assays were performed by plating 1×10^5 red cell depleted whole BM or 2×10^5 mononuclear PB cells in methylcellulose culture medium (Methocult H4435, StemCell Technologies Inc.), which contains 50 ng/ml stem cell factor (SCF), 10 ng/ml GM-CSF, 10 ng/ml IL-3 and 3 units/ml erythropoietin. After 16 days of incubation at 37 °C in a 5% CO_2 humidified incubator, all recognizable colonies were counted and the numbers combined to obtain total CFC counts as described [23]. CFC frequencies in the BM were calculated per 1×10^5 mononuclear cells. PB CFC values were converted to absolute numbers per ml of blood. The base 10 logarithm ($\log_{10}$) of these BM and PB CFC values were then used for comparisons between normal controls and the different subtypes of MDS. Blast or abnormal colonies forming clusters (<20 cells) were identified by their appearance as diffusely arranged collections of poorly refractile cells and scored as present or absent. Blast or abnormal colonies were only scored as present with confidence when $\geq$50 blast/abnormal colonies were seen in an assay [24].

2.3. Statistical analysis

A one-way ANOVA with a post hoc Bonferroni correction was performed to compare BM and PB CFC values when multiple comparisons were to be made. Linear regression analysis was performed to determine the relation between PB CFCs and WBCs. A significance level of $P < 0.05$ was chosen. To determine whether BM and PB CFC assay results from individual patients were correlated, we used R statistical programming language [25,26]. All other analyses were carried out using GraphPad Prism (GraphPad Software, San Diego, CA).

2.4. Cluster analysis

To generate heatmaps and perform hierarchical clustering, R statistical programming language was used [25,26]. The random forest (RF) classification technique was used to automatically distinguish between MDS ($n = 221$) and non-MDS (normal controls $n = 50$ and patients with systemic disease ($n = 51$)) individuals [27]. The RF classification model was determined based on the random selection of 80% of patients of each class (MDS versus non-MDS) and tested on the remaining 20% of patients. This procedure was repeated 100 times and the mean sensitivity, specificity, and positive and negative predictive values of the classification model were calculated.

3. Results

3.1. CFC values for different FAB subtypes of MDS

The total number of CFCs detected in assays of BM and (PB) blood cells from 221 patients with MDS according to the MDS subgroup to which they were assigned is depicted in Fig. 1. In comparison to normal values, significantly lower frequencies of total CFCs were seen in the BM of patients with RARS ($P < 0.01$), RAEB ($P < 0.01$) and MDS-NOS ($P < 0.01$), and absolute numbers of total PB CFCs were significantly different in MDS/MPD ($P < 0.01$) and MDS-NOS ($P < 0.05$).

When we examined specific types of progenitors, we found a significant decrease in both the primitive (BFU-E) and more mature (CFU-E) erythroid progenitors in the BM of all subgroups of MDS patients as compared to normal controls ($P < 0.05$) (Fig. 2). In the PB, a significant decrease in BFU-E was seen in patients with RA ($P < 0.01$), RAEB ($P < 0.01$), RAEB-T/AML ($P < 0.05$) and MDS-NOS ($P < 0.01$). Compared to normal, CFU-GEMMs were present at significantly lower frequencies in the BM of all MDS subgroups except MDS/MPD. CFU-GMs were also present at a significantly lower value in the marrow of patients with RAEB ($P < 0.05$) and significantly higher in the blood of patients with MDS/MPD ($P < 0.01$) compared to normal. The number of CFCs that produced abnormal/blast colonies increased with higher-risk disease (Table 1). Overall, BM assays were more sensitive than PB assays for detecting differences between normal controls and the different subtypes of MDS. Erythroid CFCs were present at consistently lower frequencies in all

Table 1
Number of patients with abnormal/blast colonies in the different subtypes of MDS.

	# of pts with abnormal BM colonies (%)	# of pts with abnormal PB colonies (%)
Normal	0/50 (0%)	0/30 (0%)
RA	10/48 (21%)	6/41 (15%)
RARS	5/24 (21%)	2/21 (10%)
RAEB	16/28 (57%)	12/22 (55%)
RAEB-T/AML	16/20 (80%)	14/17 (82%)
5q- syndrome	2/7 (29%)	2/5 (40%)
MDS/MPD	9/43 (21%)	9/42 (21%)
MDS-NOS	14/38 (37%)	12/37 (32%)