measures to account for contributions by leukocytes and red blood cells, and provided persuasive evidence that the thymosin β4 and neurogranin transcripts are derived from platelets. The significance of detecting β-globin mRNA in their preparations still needs to be resolved, and if platelet-derived, will likely require confirmation by in situ detection methods. However, Gnatenko and colleagues indirectly addressed this issue by isolating total RNA from whole blood and concluded that the globin transcripts observed in their platelet preparations were not supported by erythrocyte contaminant estimates.1

Based on serial analysis of gene expression (SAGE) results, which preferentially targets abundant mRNAs, the commentary also underscored the author’s conclusions that the vast majority of messages in platelets are mitochondrially derived.1,4 We do not argue this point. Indeed, mitochondrial RNAs are continuously transcribed, in contrast to other platelet transcripts, and generate multiple polyadenylated transcripts from individual genes accounting for their enhanced detection by SAGE.1 An important point, however, is that the mitochondrial genome encodes only 13 mRNAs and 2 rRNAs.1 Platelets contain over 2000 individual mRNA species,1,3 including well-known messages for the cytochrome c oxidase subunit 6 and β3 integrin subunits,8,9 that were not detected by SAGE.1 Thus, although they are more abundant, mitochondrially derived transcripts represent a minute fraction (<0.01%) of the mRNA species pool present in human platelets.1,3 There are numerous examples in which the identification of nonmitochondrial mRNAs in platelets has generated important physiologic insights regarding the characterization and functional significance of corresponding proteins.1,3,6,7,9,10

Although the total RNA in human platelets is at least 100-fold less than in leukocytes,10 platelet mRNAs are diverse, polyadenylated, distributed in a fashion that is influenced by cytoskeletal and RNA-binding proteins, and differentially translated in response to outside-in signals.1,3,6,8,10 In addition to mitochondrial transcripts, many others are basally present.1,3,6-10 Characterization of the platelet transcriptome, and the proteins that it encodes, will undoubtedly increase our understanding of platelet and megakaryocyte physiology and behavior in health and disease.

Andrew S. Weyrich and Guy A. Zimmerman

Correspondence: Andrew S. Weyrich, University of Utah, Department of Internal Medicine, Eccles Institute of Human Genetics, 15 North 2030 East, Bldg 533, Rm 4220, Salt Lake City, UT 84112; e-mail: andy.weyrich@hmbg.utah.edu

References


To the editor:

Does cytogenetic mosaicism in CD34+CD38low cells reflect the persistence of normal primitive hematopoietic progenitors in myeloid metaplasia with myelofibrosis?

Myeloid metaplasia with myelofibrosis (MMM) is a rare chronic myeloproliferative disorder characterized by myelofibrosis, extramedullary hematopoiensis, and absence of BCR-ABL rearrangement.1,2 Myeloproliferation is considered clonal and fibrosis, reactive.2 Hierarchic level, primary mechanism, and/or gene alteration responsible for the malignant clone remain unknown. Here, we analyzed the clonality of CD34+ cells (CD34+), and questioned the hierarchic level of the disease and the origin of karyotypically normal CD34+.

Karyotypes were performed on white blood cells (WBCs) and on immunomagnetically selected circulating CD34+ (purity, ≥97%) from 23 patients as described.3,5 According to previous reports,4,6 34.8% (8/23) of patients exhibited a high proportion of cytogenetic abnormal WBCs (nearly 100%). CD34+ carried the same cytogenetic aberrations as WBCs in patients 13, 17, 19, and 33 (Table 1), but CD34+ abnormal cell percentages were heterogeneous: 100% abnormal metaphases in patients 13, 17, and 19; 33% in patient 33; and 0% in patient 57.

This mosaicism could be due to normal residual CD34+ whose proliferation is inhibited by unknown mechanism(s).4 Normal WBC and CD34+ karyotypes in the other 14 patients strengthen the alternative hypothesis that the primitive genetic lesion remains cryptic and that karyotypic alterations occur secondarily. Mosaicism present in several CD34+ karyotypes and absent in WBCs also raises the question of the hierarchic level of the clonal event in MMM. The 6-fold higher proportion of CD34high/CD38low cells in MMM than in normal blood (25% vs 4%) suggested that the clonal myeloproliferation derives from primitive hematopoietic progenitors.1 Therefore, cytogenetic and fluorescence in situ hybridization (FISH) studies were performed on CD34+CD38low, CD34+CD38low, CD34+, and CD19+ sorted cells (99% pure) in patient 19. FISH confirmed the reciprocal translocation with monoallelic 13q14 deletion (13q14−) (lsi D13S319 probe; Vysis, Downers Grove, IL). Interestingly, in patient 19, 13q14− (FISH) was detected only in about 80% of freshly CD34+CD38low− and CD34+CD38high− sorted subpopulations (Figure 1A-B). This percentage increased after 7-day and 14-day cultures in the progeny of CD34+CD38low.
Table 1. Abnormal cytogenetic results in MMM patients: comparison of WBC, CD34* karyotypes.

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>WBC karyotypes</th>
<th>Conventional cytogenetics</th>
<th>CD34* karyotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>46, XY, t(4;12)(q22;q14)[16]/46, XY [2]</td>
<td>46, XY, t(4;12)(q22;q14)[18] (100%)</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>47, XX, t(3;20)(p22;q13), der(4)(4;10)(p26;q11), −10, +der(20)(t(3;20)(q22;q13), +mar[13]</td>
<td>47, XX, t(3;20)(q22;13), t(4;10)(q26;q11), +mar[15] (100%)</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>46, XY, t(13;17)(q14;q25)[15]</td>
<td>46, XY, t(13;17)(q14;q25)[17] (100%)</td>
<td></td>
</tr>
<tr>
<td>57</td>
<td>46, XX, del(7)(q11)[8]/46, XX [2]</td>
<td>46, XX [17]</td>
<td></td>
</tr>
</tbody>
</table>

Chromosomes were classified according to the “International System for Human Cytogenetic Nomenclature” (ISCN, 1995). Respectively, 20 or 10 normal metaphases were required in minimum to consider WBC or CD34* karyotypes as normal.

and CD38<sub>high</sub>/CD34<sup>+</sup>, suggesting a growth advantage of 13q14− cells. CD3<sup>+</sup> (T lymphocytes) and CD19<sup>+</sup> (B lymphocytes) compartments did not show the genetic marker (Figure 1C). These results contrast with those of Reeder et al<sup>8</sup> showing a heterogeneous clonal involvement (13q14−, 20q<sup>−</sup>) in MMM, at least in some patients. The absence of a cytogenetic marker in a variable percentage of CD34<sup>+</sup>, T and B cells could reflect residual normal hematopoiesis. Alternatively, karyotypically normal cells could derive from primitive progenitors/stem cells whose gene alteration(s) is cryptic.

In conclusion, we demonstrate that circulating primitive CD34<sup>+</sup>/CD38<sup>−</sup> are clonal in MMM, at least in some patients. The absence of a cytogenetic marker in a variable percentage of CD34<sup>+</sup>, T and B cells could reflect residual normal hematopoiesis. Alternatively, karyotypically normal cells could derive from primitive progenitors/stem cells whose gene alteration(s) is cryptic. Investigating these not mutually exclusive hypotheses would allow a better understanding of MMM pathogenesis and would improve its therapy.

References