Severe telomere shortening in patients with paroxysmal nocturnal hemoglobinuria affects both GPI– and GPI+ hematopoiesis
Anastasios Karadimitris, David J. Araten, Lucio Luzzatto, and Rosario Notaro

A most distinctive feature of paroxysmal nocturnal hemoglobinuria (PNH) is that in each patient glyco-osophosphatidylinositol-negative (GPI–) and GPI+ hematopoietic stem cells (HSCs) coexist, and both contribute to hematopoiesis. Telomere size correlates inversely with the cell division history of HSCs. In 10 patients with hemolytic PNH the telomeres in sorted GPI– granulocytes were shorter than in sorted GPI+ granulocytes in 4 cases, comparable in 2 cases, and longer in the remaining 4 cases. Furthermore, the telomeres of both GPI– and GPI+ hematopoietic cells were markedly shortened compared with age-matched controls. The short telomeres in the GPI– cells probably reflect the large number of cell divisions required for the progeny of a single cell to contribute a large proportion of hematopoiesis. The short telomeres of the GPI+ cells indicate that the residual hematopoiesis contributed by these cells is not normal. This epigenetic change is an additional feature shared by PNH and aplastic anemia. (Blood. 2003;102:514-516)

Study design

Subjects

Blood samples from 10 patients with hemolytic PNH (median age 32 years; range 24-60 years) and from 45 healthy individuals (median age 34 years; range 16-73 years) were obtained under Memorial Sloan-Kettering Cancer Center institutional review board–approved protocols. We included only patients with primary classical PNH who had a large PNH population (GPI– granulocyte percentage greater than 30%), florid hemoglobinuria, and no severe cytopenias (Table 1).

Immunomagnetic separation of GPI– from GPI+ granulocytes

Granulocytes, isolated as previously described,5,20 were incubated with 2 µg per 10⁶ cells of the immunoglobulin M (IgM) monoclonal antibody (mAb) anti-CD16 (Leu-1b; Becton Dickinson, Heidelberg, Germany) and then with rat anti–mouse IgM microbeads according to manufacturer instructions (Miltenyi, Bergisch Gladbach, Germany). After 30 minutes on ice, GPI– (CD16−) cells were separated from GPI+ (CD16+) cells by a
column in a strong magnetic field (MACS; Miltenyi). All separation steps were carried out strictly at 4°C.

Telomere length measurement

Telomere length (TRF) measurement was carried out by a method we have previously developed based on a probe, TelBam8, that is unique for the subtelomeric region of the long arm of chromosome 7.

Statistical analysis

All data are expressed as mean ± SD. The expected-for-age telomere length (TRF₀) has been estimated by linear regression of TRF against age of 45 healthy individuals: TRF₀ = 18.352 bp − 53 bp × age (years) (R² = 0.12; P = .02). The 53-bp-per-year TRF loss is in agreement with previous reports. Wilcoxon rank sum test on paired samples, Kendall correlation, and Fisher exact test have been used when appropriate. Statistical significance was accepted for P < .05.

Results and discussion

In order to study the dynamic relationship between the clonal GPI⁻ hematopoiesis and the coexisting residual GPI⁺ hematopoiesis in each PNH patient, we compared the telomere length of both these populations from 10 patients with classical hemolytic PNH (Table 1). We measured side by side in each patient the telomere length of purified GPI⁻ and GPI⁺ peripheral blood granulocytes (Figure 1A). Being terminally differentiated cells, these are likely to reflect the replicative history of HSCs. This internally controlled comparison showed that, overall, the average telomere length was similar in GPI⁻ and GPI⁺ granulocytes: 13.9 ± 0.9 kilobase (kb) versus 13.8 ± 0.9 kb; P = .64 (Figure 1B–C). Nevertheless, we found different patterns in individual patients. The telomeres of GPI⁻ granulocytes, compared with those of GPI⁺ granulocytes, were longer in 4 patients, similar in 2 patients, and shorter in the remaining 4 patients. We have investigated how these patterns relate to the patients’ hematologic and biologic characteristics (Table 1). We have found that a difference in telomere length in favor of GPI⁺ granulocytes correlates directly with the size of the PNH cell population (P = .032).

Next, we compared the telomere length of granulocytes from PNH patients with that from healthy individuals. As telomere length is extremely variable in the population, we resorted to calculating the difference between the telomere length observed (TRF₀) in each individual and the expected-for-age telomere length (TRF₀): TRF₀ = 140 ± 1935 bp) and symmetrically distributed around the zero point (Figure 1D). By contrast, in both GPI⁻ and GPI⁺ granulocytes from PNH patients, all but one TRF₀ values were below zero (TRF₀⁻: GPI⁺, −2563 ± 1306 bp; GPI⁻, −2730 ± 1256 bp; Figure 1D), and both TRF₀⁻ distributions were significantly different from that in healthy individuals (healthy vs GPI⁺: P = .011; healthy vs GPI⁻: P = .001).

Thus, we have found in this study that both the GPI⁻ and the GPI⁺ blood cells from patients with classical hemolytic PNH have shorter telomeres than blood cells from age-matched healthy individuals. Assuming that telomeres lose approximately 100 bp per cell division, the telomere shortening observed in PNH patients would be equivalent to approximately 25 extra cell divisions. The extreme telomere shortening in the GPI⁺ blood cells is in keeping with the fact that in hemolytic PNH patients, just 1 clone, or a handful of clones supports a substantial proportion of hematopoiesis. It stands to reason that in order to achieve this task, which entails substantial expansion, these HSCs must perform a considerable number of extra mitotic divisions.

As for the shortening of telomeres found in the GPI⁺ blood cells, it is reminiscent of that reported in AA patients (it may be in fact even greater), indicating that in PNH patients the residual GPI⁻ hematopoiesis is not normal. A hypothetical explanation is that selective destruction of normal (GPI⁺) HSCs has left very few survivors. These must once again expand to support the residual GPI⁻ hematopoiesis in PNH patients. This hypothesis could also explain why different patterns are seen in different patients. This
Table 1. Clinical and hematologic features of PNH patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age</th>
<th>Years from diagnosis</th>
<th>Hb, g/L</th>
<th>PMNs, $10^9$/L</th>
<th>Plts, $10^9$/L</th>
<th>Retics, %</th>
<th>Size of PNH population, %*</th>
<th>Absolute counts</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>F/24</td>
<td>6</td>
<td>101.2</td>
<td>23.1</td>
<td>130.0</td>
<td>4.5</td>
<td>39.80</td>
<td>0.46 1.84</td>
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<tr>
<td>B</td>
<td>F/27</td>
<td>7</td>
<td>108.4</td>
<td>4.2</td>
<td>115.0</td>
<td>3.9</td>
<td>30.90</td>
<td>0.42 3.78</td>
</tr>
<tr>
<td>C</td>
<td>M/40</td>
<td>2</td>
<td>118.2</td>
<td>3.2</td>
<td>137.0</td>
<td>3.4</td>
<td>13.32</td>
<td>2.18 1.02</td>
</tr>
<tr>
<td>D</td>
<td>M/30</td>
<td>3</td>
<td>90.2</td>
<td>2.6</td>
<td>180.0</td>
<td>14.9</td>
<td>43.94</td>
<td>0.16 2.44</td>
</tr>
<tr>
<td>E</td>
<td>F/37</td>
<td>7</td>
<td>82.1</td>
<td>1.9</td>
<td>115.0</td>
<td>8.2</td>
<td>12.59</td>
<td>0.78 1.12</td>
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<tr>
<td>F</td>
<td>F/24</td>
<td>5</td>
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<td>3.1</td>
<td>103.0</td>
<td>3.0</td>
<td>60.80</td>
<td>0.62 2.48</td>
</tr>
<tr>
<td>G</td>
<td>F/34</td>
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<td>74.0</td>
<td>2.1</td>
<td>23.40</td>
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<tr>
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<td>160.0</td>
<td>5.2</td>
<td>18.77</td>
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<tr>
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<td>106.8</td>
<td>6.4</td>
<td>62.0</td>
<td>5.4</td>
<td>72.96</td>
<td>0.34 8.06</td>
</tr>
<tr>
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<td>F/60</td>
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<td>70.3</td>
<td>3.0</td>
<td>229.0</td>
<td>ND</td>
<td>46.78</td>
<td>0.66 2.34</td>
</tr>
</tbody>
</table>

Hb indicates hemoglobin level; PMNs, granulocytes; Plts, platelets; Retics, reticulocytes; RBCs, red blood cells; Yes, recurrent episodes of macroscopic hemoglobinuria throughout several years of clinical history; F, female; M, male; ATG, antithymocyte globulin; and ND, not done.

*The percentage of PNH PMNs reflects the relative size of the PNH cell population more accurately than the percentage of PNH RBCs because the latter will be grossly underestimated as a consequence of selective hemolysis; this underestimation may be further complicated by blood transfusion.

may depend on how far the PNH clone(s) has expanded at a particular point time, on whether damage to GPI$^+$ cells is still on-going, etc. Indeed, telomeres being shorter in GPI$^+$ than in GPI$^-$ granulocytes in patients with a larger PNH cell population may suggest that, because of an on-going damage, GPI$^+$ HSCs are less able to contribute to hematopoiesis. Thus, telomere shortening could be regarded as an epigenetic marker of disease and one more feature that is shared by PNH and AA patients.

In summary, we have shown that severe telomere shortening affects GPI$^+$ and GPI$^-$ hematopoiesis in patients with PNH roughly to the same extent. However, it is likely that these apparently similar epigenetic changes are caused by somewhat different mechanisms such as clonal expansion in the GPI$^+$ HSC and oligoclonal regeneration after selective destruction in the GPI$^+$ HSC.

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References