Brief report

Clonal hematopoiesis in familial polycythemia vera suggests the involvement of multiple mutational events in the early pathogenesis of the disease

Robert Kralovics, David W. Stockton, and Josef T. Prchal

Familial clustering of malignancies provides a unique opportunity to identify molecular causes of cancer. Polycythemia vera (PV) is a myeloproliferative disorder due to an unknown somatic stem cell defect that leads to clonal myeloid hyperproliferation. We studied 6 families with PV. The familial predisposition to PV appears to follow an autosomal dominant inheritance pattern with incomplete penetrance. All examined females informative for a transcriptional clonality assay had clonal hematopoiesis. We excluded linkage between PV and a number of previously proposed candidate disease loci (c-mpl, EPOR, 20q, 13q, 5q, 9p). Therefore, mutations at these loci are unlikely primary causes of familial PV. The finding of erythropoietin-independent erythroid progenitors in healthy family members indicated the presence of the PV stem cell clone in their hematopoiesis. This finding, together with clonal hematopoiesis in the affected individuals, supports the hypothesis of multiple genetic defects involved in the early pathogenesis of PV.

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Results and discussion

We studied 6 white families of heterogeneous ethnic background, each with multiple members with PV (Figure 1A). We detected EECs in peripheral blood cultures in all the affected family members in all families. The clinical findings are summarized in Table 1; these results of clinical findings represent all of the available patients and these were not preselected. In addition to the affected members with the full PV phenotype, we tested family members without PV for the presence of EECs in their peripheral blood. We identified subjects in families A and B who had no clinical signs or symptoms of PV but EECs were present in their peripheral blood (subjects A04, A06, A09, A14, A17, B01). Clonality was demonstrated in the informative females with full PV phenotype (Table 1; Figure 1B). The members with partial phenotype (EECs only) were polyclonal, indicating the contribution of normal stem cells to productive hematopoiesis, and therefore, lack of clinical symptoms of PV. To examine whether the presence of EECs is due to a somatic mutation or an inherited mutant gene, we isolated the Epo-independent erythroid cell clone in their hematopoiesis. This finding, together with clonal hematopoiesis in the affected individuals, supports the hypothesis of multiple genetic defects involved in the early pathogenesis of PV.

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Study design

All affected family members had classical diagnosis of PV based on the PV Study Group criteria. All studies were performed under approved institutional review board protocols (Baylor College of Medicine and University of Alabama at Birmingham), and all subjects included in this study provided written consent to perform DNA and cell culture studies on their blood samples.

Detailed description of the EEC assay, cell isolations, clonality analysis, loss of heterozygosity (LOH) detection, and linkage analysis are listed elsewhere. Microsatellite polymerase chain reaction for the EPOR and c-mpl genes was done as previously described.

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hematopoiesis; please note that the T cells’ X-chromosome allelic usage appears skewed; however, the observed skewing is within the range that we reported in normal hematopoietic progeny (Figure 1B).16

The inheritance pattern of familial PV is compatible with an autosomal dominant trait with decreased penetrance. If the finding of EECs is considered as an early sign of the PV phenotype, the penetrance increases. Of the 6 families, 4 (A-D) were used for linkage analysis since these consisted of at least 2 affected siblings. It is possible that the clustering of PV in families E and F could be by chance only and that we could have had a selection bias to recruit these families since families of unusual polycythemic disorders have been referred to us for more than a decade. For these pedigrees, we calculated the power to detect linkage using the SIMLINK software (http://www.sph.umich.edu/group/statgen/software),17 which predicted a maximum logarithm of odds ratio (LOD) score of 4.4 for the given pedigree structures. The simulated LOD score further increased to 5.2 when the subjects positive for EECs were considered as affected (data not shown). Using these families, we could examine a number of candidate loci that were previously proposed to play a role in MPD, or PV in particular. We analyzed the linkage between the PV phenotype and the commonly deleted regions on chromosomes 20q, 13q, and 5q that were found as genetic aberrations in PV.18,19 In addition, the thrombopoietin

**Figure 1. Analyses of 6 families with PV.** (A) Pedigrees of 6 families with polycythemia vera (PV). Filled symbols indicate affected individuals. In families A and B, black dots indicate hematologically normal family members with Epo-independent erythroid cells detectable in their peripheral blood cultures. (B) Clonality analysis of individuals A01 and A06 using the IDS gene exonic polymorphism. RNA isolated from T lymphocytes (T), granulocytes (GRA), platelets (PLT), and Epo-independent erythroid cells (EEC) were used for the analysis. The presence of only one expressed allele of the IDS gene in platelets, granulocytes, and Epo-independent erythroid cells is consistent with clonal origin of cells. (C) Exclusion of linkage between chromosome 9p microsatellite markers and PV. LOD scores less than –2.0 satisfy the criteria of exclusion of linkage. The dotted line depicts the customary evidence for genetic exclusion of linkage. DM indicates centimorgan.

### Table 1. Summary of clinical findings

<table>
<thead>
<tr>
<th>Subject</th>
<th>Diagnosis</th>
<th>Sex</th>
<th>Age at diagnosis, y</th>
<th>Hct</th>
<th>Hgb, g/L</th>
<th>PLT, K/μL</th>
<th>Spleenomegaly</th>
<th>Karyotype</th>
<th>Clonality assay</th>
<th>EEC</th>
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<tr>
<td>A01</td>
<td>PV</td>
<td>F</td>
<td>58</td>
<td>.55</td>
<td>180</td>
<td>443</td>
<td>+</td>
<td>9pLOH</td>
<td>CL</td>
<td>Present</td>
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<tr>
<td>A03</td>
<td>PV</td>
<td>F</td>
<td>62</td>
<td>.64</td>
<td>197</td>
<td>190</td>
<td>+</td>
<td>Normal</td>
<td>CL</td>
<td>Present</td>
</tr>
<tr>
<td>A04</td>
<td>Normal</td>
<td>F</td>
<td>—</td>
<td>.422</td>
<td>147</td>
<td>310</td>
<td>–</td>
<td>ND</td>
<td>ND</td>
<td>Present</td>
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<tr>
<td>A06</td>
<td>Normal</td>
<td>F</td>
<td>—</td>
<td>.467</td>
<td>162</td>
<td>204</td>
<td>–</td>
<td>ND</td>
<td>PO, EEC-CL</td>
<td>Present</td>
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<tr>
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<td>PV</td>
<td>F</td>
<td>31</td>
<td>.457</td>
<td>127</td>
<td>438</td>
<td>–</td>
<td>9pLOH</td>
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<td>M</td>
<td>33</td>
<td>.484</td>
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<td>ND</td>
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<tr>
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<td>F</td>
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<td>.542</td>
<td>177</td>
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<tr>
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<td>ND</td>
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<tr>
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<td>M</td>
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<td>910</td>
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<tr>
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<tr>
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<tr>
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<td>52</td>
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<tr>
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<td>.50</td>
<td>164</td>
<td>168</td>
<td>–</td>
<td>Normal</td>
<td>CL</td>
<td>Present</td>
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</table>

Hct indicates hematocrit; Hgb, hemoglobin; CL, clonal; —, not present; ND, not determined; PO, polyclonal; and EEC, Epo-independent colonies.
receptor (c-mpl) and Epo receptor (EPOR) genes have been proposed in the pathogenesis of myeloproliferative disorders including PV. Recently, we reported the presence of LOH involving chromosome 9p as the most common clonal defect in sporadic PV. We detected LOH on chromosome 9p in subjects A01 and B02 (Table 1). We performed linkage analysis using microsatellite markers mapping to these loci. Only the affected subjects with the full PV phenotype were considered “affected” in the linkage analysis. Since LOD scores less than −2.0 are considered exclusion of linkage, we could exclude linkage between the PV phenotype and all the tested loci. LOD scores for the EPOR and c-mpl genes were −3.16 and −2.24, respectively. The commonly deleted regions found in sporadic MPD were also convincingly excluded with LOD scores of −4.40 for 20q, −4.79 for 13q, and −6.27 for 9q. We also fully excluded the chromosome 9p region of LOH (Figure 1C). These results suggest that the frequently observed somatic mutations in MPD involving the chromosomal regions on 20q, 13q, 9q, and 9p are secondary genetic changes and do not target the primary PV locus.

The clinical analysis of the affected family members confirmed that they are phenotypically identical to sporadic PV. Familial occurrence of PV provides a unique insight into the stages of PV since we could identify affected members in an early stage of the disease using the EEC assay. This is not possible in sporadic PV since individuals are identified only when symptomatic. Thrombocytopenia was shown to be the first abnormality seen in some PV subjects, and interestingly in families C and D, thrombocytopenia occurred prior to elevation of hematocrit. In all the affected informative females, we observed clonal circulating myeloid cells as seen in sporadic PV; in some, clonality could not be determined because (1) patients were genotyped for exonic polymorphisms of active X-chromosome genes used for the clonality assays and were not informative, (2) there was failure to get informed consent for this follow-up study, or (3) patients were no longer available or willing to participate in our study. Clonal hematopoiesis is a marker of fully developed PV. In families A and B, we observed hematologically normal subjects with EECs present in their peripheral blood. In one of these cases we proved the clonal origin of the Epo-independent cells, but all the rest of the myeloid cells were polyclonal. Thus, it is possible that in presymptomatic PV within polyclonal hematopoiesis the PV stem cell clone may be present but its contribution to blood production is limited. At this stage, the progeny of the PV clone can be detectable by the EEC assay. In the symptomatic stage, the PV clone loses regulation and expands, and clonal hematopoiesis is evident in peripheral blood. In one of these cases we proved the clonal origin of the Epo-independent cells, but all the rest of the myeloid cells were polyclonal. Thus, it is possible that in presymptomatic PV within polyclonal hematopoiesis the PV stem cell clone may be present but its contribution to blood production is limited. At this stage, the progeny of the PV clone can be detectable by the EEC assay. In the symptomatic stage, the PV clone loses regulation and expands, and clonal hematopoiesis is evident in peripheral blood.

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References

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