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Detection of abnormalities of *PRV-1*, *TPO*, and *c-MPL* genes detected by fluorescence in situ hybridization in essential thrombocythemia

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Abstract No specific diagnostic markers have been described in essential thrombocythemia (ET). *PRV-1* (polycythemia rubra vera-1), *TPO* (thrombopoietin), and *c-MPL* (myeloproliferative leukemia virus oncogene) genes are candidate ET molecular markers because of their implication in the pathogenesis of ET. We have studied the status of *PRV-1*, *TPO*, and *c-MPL* genes in 30 ET patients by a fluorescence in situ hybridization (FISH) technique using three noncommercial, locus-specific probes for *PRV-1* (BAC RP11-160A19, located at 19q13.2), *TPO* (BAC RP11-45NP16, located at 3q27), and *c-MPL* (BAC RP11-297L5, located at 1p34). FISH study showed no *PRV-1*, *TPO*, and *c-MPL* cytogenetic abnormalities in any of the analyzed cases. Our results suggest a lack of structural and numerical rearrangements (deletions, translocations, or amplifications) of *PRV-1*, *TPO*, and *c-MPL* genes in ET patients.

1. Introduction

Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) with heterogeneous features and no specific diagnostic markers. As a result, its diagnosis is based on the exclusion of other CMPD and secondary thrombocytosis. The study of molecular alterations has focused on the *PRV-1* (polycythemia rubra vera-1), *TPO* (thrombopoietin), and *c-MPL* (myeloproliferative leukemia virus oncogene) genes because of their implication in ET pathogenesis [1].

The *PRV-1* gene is a cell-surface receptor that belongs to the Ly-6/uPAR superfamily [2]. Several groups have published *PRV-1* gene overexpression in granulocytes from polycythemia vera (PV) and in some ET patients, but not in secondary erythrocytosis (SE), in chronic myeloid leukemia (CML), nor in healthy individuals. Therefore, the quantification of *PRV-1* mRNA represents a specific molecular marker for PV (it discriminates between PV and SE), but not for ET [3]. *TPO* and its receptor, *c-MPL*, regulate megakaryocytic proliferation and differentiation. *c-MPL* expression in megakaryocytes and platelets is generally decreased in ET but it may be also decreased in reactive thrombocytosis [4] and in hereditary trombocythemia [5]. Its diagnostic value is limited but it would be useful as a prognostic marker for its correlation with thrombotic events [6,7].

Regarding gene status studies, the absence of *PRV-1* anomalies detected by fluorescence in situ hybridization (FISH) has been reported in PV [8], but there are no results for *PRV-1*, *TPO* and *c-MPL* genes in ET patients.

The aim of this study was to analyze the status of *PRV-1*, *TPO*, and *c-MPL* genes by FISH to find molecular markers in ET patients.

2. Patients and methods

2.1. Patients

Thirty bone marrow samples from patients diagnosed with ET following the Polycythemia Vera Study Group (PVSG) criteria [9] and 10 bone marrow samples from healthy individuals, which were used as assay validation

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controls, were included in the study. All patients (7 males and 23 females) came from Hospital del Mar in Barcelona, and the diagnoses were established between 1988 and 2004.

2.2. FISH technique

FISH studies were performed on fixed nuclei from bone marrow samples proceeding from conventional cytogenetics cultures. Chromosome analyses had been performed previously on hematologic cells from 24-hour bone marrow cultures, and all cases showed a normal karyotype [10]. All samples were studied using three noncommercial, locus-specific probes (BAC) for *PRV-1*, *TPO*, and *c-MPL* genes as follows: (1) *PRV-1* gene [BAC RP11-160A19, 157 kilobases (Kb), located at 19q13.2] labeled in SpectrumRed-dUTP cohybridized with the 19p telomeric probe (D19S238E; Vysis, Downers Grove, IL) labeled in SpectrumGreen (Fig. 1A); (2) the *TPO* gene (BAC RP11-45NP16, 183 Kb, located at 3q27) labeled in SpectrumGreen-dUTP cohybridized with the centromeric probe for chromosome 3 (D3Z1; Vysis) labeled in SpectrumRed (Fig. 1B); and (3) the *c-MPL* gene (BAC RP11-297L5, 190 Kb, located at 1p34) labeled in SpectrumGreen-dUTP cohybridized with the centromeric probe for chromosome 1 (D1Z5; Vysis) labeled in SpectrumOrange (Fig. 1C).

The selected BACs were obtained from the CHORI BAC/PAC resource (http://bacpac.chori.org). Clones were grown using standard procedures and BAC DNA was extracted using standard alkaline lysis techniques (Qiagen GmbH, Hilden, Germany). Probe DNA was labeled by nick translation (Vysis) as indicated by the manufacturer. The cytogenetic localization of all probes was verified by hybridization to normal metaphase chromosomes (G-banding with inverted DAPI).

Regarding the hybridization process, slides were denatured for 5 minutes at 70°C in a 70% formamide solution

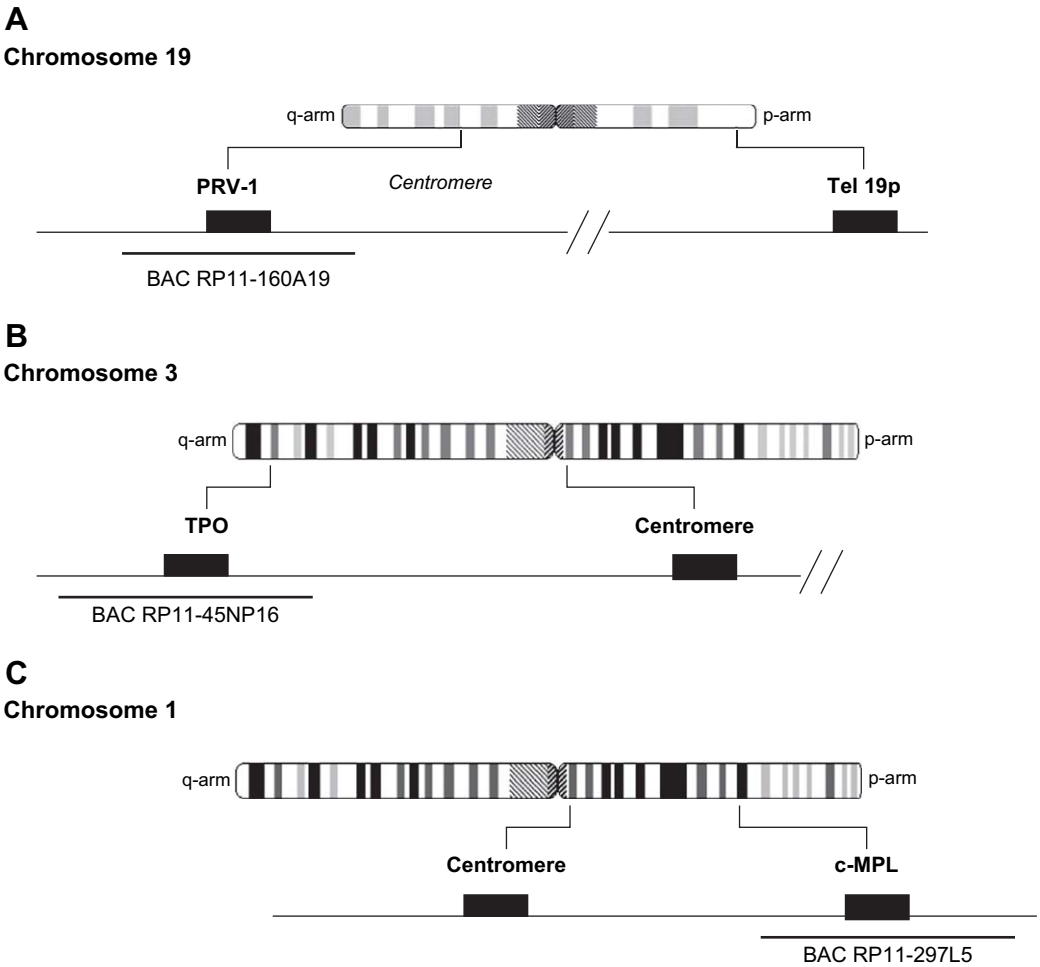

Fig. 1. Physical maps for the DNA probes used in this project. (A) Probe for the *PRV-1* gene (BAC RP11-160A19, 157 Kb), located at 19q13.2, labeled in SpectrumRed-dUTP cohybridized with the 19p telomeric probe (D19S238E; Vysis), labeled in SpectrumGreen. (B) Probe for the *TPO* gene (BAC RP11-45NP16, 183 Kb), located at 3q27, labeled in SpectrumGreen-dUTP cohybridized with the centromeric probe for chromosome 3 (D3Z1; Vysis), labeled in SpectrumRed. (C) Probe for *c-MPL* gene (BAC RP11-297L5, 190 Kb), located at 1p34, labeled in SpectrumGreen-dUTP cohybridized with the centromeric probe for chromosome 1 (D1Z5; Vysis), labeled in SpectrumOrange. Map not drawn to scale