The Myeloperoxidase Gene in Acute Promyelocytic Leukemia

Acute promyelocytic leukemia (APL) is a subtype of leukemia with a specific cytogenetic translocation [t(15;17)(q22;q11.2)]. These cells express abundant myeloperoxidase, an enzyme that catalyzes the synthesis of hypochlorous acid, which contributes to the microbicidal function of granulocytes.

Weil et al. (1) report the mapping of the myeloperoxidase gene (MPO) to human chromosome 17q12–q21, the region of the breakpoint on chromosome 17 in APL. The chromosomal localization of MPO at bands q11–q21, on the basis of four published studies mapping MPO to chromosome 17 on bands q21–q23 or q22–q24, several million base pairs away from the breakpoint of APL on chromosome 17 (2, 3). Chromosomal localization by in situ hybridization is generally less accurate than screening a panel of somatic cell hybrids containing fragments of chromosome 17. Using such a panel, van Tuinen et al. ruled out localization of MPO to 17q12–q21 (2).

In support of the more distal location of MPO on 17q reported by both van Tuinen et al. (2) and Chang et al. (3), we now know that a number of genes intervene between the APL breakpoint and the MPO locus. Loci for NGFR (4), EMOB3 (5), HOX2 (6), and GFAP (glial fibrillar acidic protein) mapped by W. Westmark and colleagues in collaboration with one of us (K.F.H.) are distal to the APL breakpoint but proximal to the MPO locus (7). The observation by Weil et al. (1) that MPO, normally located on chromosome 17q, was translocated to chromosome 15 in APL is therefore not surprising and supports the conclusions of others (2, 3, 8).

Further, Weil et al. (1) present evidence from Southern blotting of rearrangement of MPO in DNA from bone marrow of two of four patients with APL. The data suggest a high frequency of rearrangement of the transcriptionally active MPO in APL and could represent an important step in understanding the etiology of the disease.

To determine the incidence of rearrangement of MPO in APL, DNA samples from bone marrow of 13 patients with APL were digested with Bam HI and Bgl II, electrophoresed, Southern blotted, and hybridized with several 32P-labeled cDNAs coding for MPO (Fig. 1). No novel bands were detected, while all normal bands identified by Weil et al. (1) were detected. We conclude that rearrangement of MPO is at most an infrequent occurrence in APL. DNA from three patients with APL was subjected to inverted field gel electrophoresis after digestion with infrequently cutting restriction endonuclease. In no case could rearrangements of the gene be detected within 1000 kb of the coding region.

Because of our inability to confirm the findings of Weil et al. (1), we examined the characteristics of the two MPO cDNA probes used in their study. Their two recombinant plasmids, pHPM10A and pHMP2E, cover an apparent ~2 kb at the 3′ end of the longest (3.3 kb) of the two RNAs that code for MPO. These probes have not been sequenced, but the authors state that they have compared their restriction maps with the sequence of the near full-sized clone that we have documented to be MPO (9). Their restriction map in the 5′ end of clone pHMP10E and in the 3′ end of clone pHMP2E is different from the map that can be derived from the identical MPO cDNA sequence data of Johnson et al. (9) and Morishita et al. (10) (Fig. 2). The relevant differences are that the Bgl II site at the 5′ end of pHMP10A does not exist in the published sequences; however, a Kpn I site found in this region, as deduced by sequencing data, is missing in their clones. The restriction map of the 3′ half of pHMP2E clone is completely different from the maps deduced by the sequencing data. Furthermore, clone pHMP2E extends 600 base pairs further in the 3′ direction than any reported sequence. We therefore suspect, barring trivial mistakes on our part in generating their restriction map, that Weil et al. have either cDNAs that are the result of a cloning artifact or that MPO contains an extremely high degree of polymorphism.

Furthermore, the results as presented by Weil et al. (1) do not prove that MPO is rearranged in APL for several reasons: (i) In one of their APL samples (L2), they found an extra band using only one of three restriction enzymes (Bgl II), which does not rule out an infrequent restriction fragment length polymorphism (RFLP). The extra Bgl II sites present in their cDNA clones, if real, would suggest an RFLP. (ii) The genomic restriction map of MPO (11) suggests that the rearrangements which introduced new sites for Bgl II in one of their samples.

**Fig. 1.** Restriction pattern of MPO in DNA of bone marrow cells from 13 patients with acute promyelocytic leukemia and DNA of normal human fibroblasts (C). The DNA from patients was derived from a population of cells that contained more than 80% APL cells. The leukemic cells of each patient contained the typical t(15;17) of APL. Samples were digested with Bgl II and probed with the Kpn I–Hind III 0.5-kb fragment of pMP02 (9), a 3′ specific probe. Digestion, electrophoresis, Southern blotting, and hybridization were performed by standard techniques (10). Gel-purified fragments were 32P-labeled by random priming, to 106 cpm/μg (11). The same DNAs were also digested with Bam HI and hybridized sequentially with the same probe and with 32P-labeled pMP062, a full-length cDNA (9). The restriction pattern of DNA samples from the patients was identical to that from normal fibroblasts.

**Fig. 2.** Restriction maps of putative MPO cDNA clones. Maps (A) and (B) are those reported by Weil et al. (1). Map (C) is computer generated from the 3′ region of cDNA sequence data of Johnson et al. (9) and of Morishita et al. (10). Restriction endonuclease cleavage sites are Bgl II (B), Kpn I (K), Pst I (P), Pvu II (PV), and Sma I (S).
and for Hind III and Bgl II in the other would affect the size of other restriction fragments in their restriction digests. These were not observed. (iii) One or several novel restriction fragments occurred in the DNA from one of their APL patients with an MPO rearrangement (L1) when the patient was in remission; theoretically the restriction pattern should have been normal or at least the same as the pattern during active disease.

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Acute promyelocytic leukemia (APL) is consistently associated with a reciprocal translocation involving chromosomes 15 (band q22) and 17 (band q11–q12) (1). The DNA segments directly involved in this translocation, t(15;17), have not yet been identified and, as a result, its role in the pathogenesis of APL is not yet determined.

Fig. 1. Distribution of MPO-hybridizing sites on normal human chromosome 17. In the 105 metaphases analyzed, 21% of 148 grains were observed on the long arm of chromosome 17 (P < 0.001) with 75% of grains on band 17q22-q23.

Weil et al. (2) report that the myeloperoxidase (MPO) gene (MPO) maps to human chromosome 17 (q12–q21) and that it is rearranged and translocated to chromosome 15 in cases of APL. The implications of these results, if correct, are important to an understanding of the pathogenesis of APL, as they provide precise information about the location of the breakpoint on chromosome 17 and indicate the molecular strategy for identifying critical DNA sequences on the recombinant chromosome 15.

As part of more extensive research aimed at molecularly characterizing the t(15;17), we also mapped MPO to normal human chromosomes and determined the position of the breakpoint on chromosome 17 with respect to MPO in cases of APL. However, our findings do not agree with those reported by Weil et al.

For chromosomal localization of MPO, we used a nearly full-length cDNA probe representative of the human MPO gene [clone pMP062, a gift from G. Rovera; see (3)]. Labeled pMP062 DNA was hybridized to chromosome preparations from normal peripheral blood lymphocytes. The MPO probe hybridized to chromosomes 17, and silver grains were consistently located on band q22–q23, as has been previously reported (4) (Fig. 1). We conclude that MPO maps to a region distal to the chromosomal region 17q11–q12 involved in the t(15;17).

To investigate whether parts of the MPO gene or its immediately flanking sequences were directly affected by the breakpoint on chromosome 17, we performed Southern blot analysis of genomic DNA from 27 cases of APL. Diagnosis of APL was formulated according to classical, morphological, cytochemical, and immunophenotypic criteria in all cases. Cytogenetic data, which were available for 13 cases, revealed the presence of a typical t(15;17) in all cases. DNAs were digested with Bam HI, Bgl II,
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