Correspondence

To the editor:

Intron 1 factor VIII gene inversion in a population of Italian hemophilia A patients

Bagnall and colleagues\(^1\) report a technique to investigate an inversion that disrupts the factor VIII (F8) gene and that represents a frequent cause of severe hemophilia A. This large genomic rearrangement, identified for the first time by Brinke et al\(^2\) in 2 haemophilic monozygotic twins, affects intron 1 of the F8 gene. Bagnall et al\(^3\) demonstrated that this inversion of intron 1 derives from a homologous recombination between 2 nearly identical 1041-base pair (bp) sequences, int1h-1 and int1h-2, in opposite orientation, positioned respectively in intron 1 of the gene and in a more telomeric region, 140 kilobases (kb) downstream, between the C6.1A and VBP1 genes. This rearrangement results in the production of 2 chimeric mRNAs; the first, possibly under the control of the F8 gene promoter, contains the first exon of the F8 gene, followed by some exons of VBP1. The other chimeric mRNA, under control of the C6.1A gene promoter, contains almost all of the coding region of the C6.1A gene followed by part of intron 1 and exon 2 to 26 of the F8 gene.

We have examined 28 patients with severe to moderate hemophilia A (21 with severe, 2 with moderately severe, and 5 with moderate hemophilia A phenotype). All of the patients were investigated for the inversion of intron 223-4; 8 of them are characterized by the presence of this common mutation. The other affected males that were negative for the inversion of intron 22 were screened for the inversion of intron 1; this mutation occurs repeatedly and seems to have a prevalence of 5% in patients with severe disease in the United Kingdom.

We have performed an analysis directly on genomic DNA extracted with a QIAamp DNA blood mini-kit (QIAGEN, Hilden, Germany) under polymerase chain reaction (PCR) conditions suggested by Bagnall et al\(^1\). We performed 2 amplifications. One reaction contained 3 different primers: 9F and 9cR, specific for the int1h-1 region, and int1h-2F, which is specific for the region flanking int1h-2. The other reaction contained 2 primers, int1h-2F and int1h-2R, which are specific for the amplification of the int1h-2 region, and a third primer, 9F, which flanks the telomeric side of the int1h-1 region. These PCRs are able to differentiate wild type from the patients with inversion and the patients with inversion from carriers. The amplified segments were readily separated on a 1.5% agarose gel. One patient out of 20 with severe disease tested positive for intron 1 inversion (Figure 1).

These observations demonstrate, in accordance with Bagnall et al, that this approach is a rapid and efficient method for the individuation of intron 1 inversion and it should be routinely performed in patients with severe hemophilia A.

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To the editor:

Lack of \(p21^{CIP1}\) DNA methylation in acute lymphocytic leukemia

Recently, Roman-Gomez et al\(^1\) reported an incidence of \(p21^{CIP1}\) methylation of 41% in 124 patients with acute lymphocytic leukemia (ALL). Most importantly, they observed that \(p21^{CIP1}\) methylation was an independent predictor of poor prognosis both in adults and children with this disease.

To follow these observations, we have analyzed the methylation status of \(p21^{CIP1}\) in a cohort of patients with ALL who were previously studied for methylation of multiple genes.\(^2,3\) We studied a total of 31 patients (19 male; median age, 39 years [range, 7-77 years]; 6 Philadelphia (Ph) chromosome positive). For methylation analysis, we used widely accepted methods based on bisulfite modification of DNA because these assays are sensitive and have a low rate of false positivity. We used 2 different bisulfite polymerase chain reaction (PCR) methods to assess methylation of 3 different
regions in or in close proximity to the area studied by Roman-Gomez et al. Primer location is shown in Figure 1. The methylation status of region 1 was analyzed using the combined bisulfite restriction analysis assay (COBRA). A positive control consisting of genomic DNA methylated in vitro by SssI methylase was used in all of the assays to verify the validity of the procedures. Using these 2 techniques, DNA methylation was not observed in any of the samples studied in any of the 3 regions analyzed (Figure 1). In contrast, methylation of many other genes, including ER, MDR1, THBS1, THBS2, p15, p73, Myf3, c-abl, and CD10, was observed previously in some of these cases. To analyze p21 expression status, we have performed reverse transcriptase (RT)–PCR analysis in 8 cases. In contrast to the results reported by Roman-Gomez et al, all the cases studied had evidence of p21 methylation (Figure 1).

Our results are in contrast with those of Roman-Gomez et al. No other investigator has reported evidence of p21 methylation in ALL or other leukemias. Indeed, Kikuchi et al did not find evidence of p21 methylation in 19 neoplastic cell lines, including 6 of hematopoietic origin. It is possible that the patients studied by Roman-Gomez et al are markedly different from patients in the United States, as geographic variation in methylation patterns has recently been reported. However, we suggest that the differences observed reside in the technique used by Roman-Gomez et al to assess methylation. In their experiments, they digested DNA with a methylation-sensitive restriction enzyme followed by PCR amplification. Unrestricted PCR-amplified fragments thus represented methylated alleles. Because all cases had evidence of an amplified (methylated) band, samples were considered methylated based on a normalized mean ratio of p21 expression to β-actin PCR amplification. This method is prone to false-positive results because unmethylated but incompletely digested DNA may amplify and give a positive reading. The authors did not show controls, nor did they confirm the methylation status of p21 by Southern blot analysis or bisulfite-based methods. These 2 last methods are considered more reliable.

In summary, we have found no evidence of p21 methylation in our cohort of patients. Other investigators have reported similar findings in an extensive study of neoplastic cell lines. We believe that Roman-Gomez et al should reanalyze their samples using either Southern blot or bisulfite-based methods to confirm their important observations.

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References

Response:

Hypermethylation of the p21 gene in acute lymphoblastic leukemia

Shen et al analyzed the methylation status of the p21 gene in a cohort of ALL patients using methods based on bisulfite modification of DNA. They found lack of p21 methylation in their patients. This result is in disagreement with our recent report. However, the study by Shen et al contains a number of weak points and several problems.

First, their study included 31 acute lymphocytic leukemia (ALL) patients (probably selected by the availability of preserved DNA or other unknown reasons), which is a very small number of patients when the goal is to reach a definitive conclusion regarding the frequency of this molecular event. By comparison, our study included 124 consecutive ALL patients. It has been suggested that there are important geographic variations in the methylation patterns of several malignancies. For example, in our series of 150 consecutive Spanish ALL patients (manuscript in preparation), we have observed methylation of the E-cadherin and p73 genes in 19% and 18% of patients respectively, whereas E-cadherin methylation has been observed in 53% of patients in the United States and in
76% of patients in Australia.2-3 By the same token, p73 methylation has been detected in 32% of patients in the United States.1-5 However, we think that these differences do not depend only on geographic variations but also on the premature release in high-impact journals of a plethora of descriptive methylation studies based on small and very selected groups of patients. These preliminary reports have reached conclusions that have been accepted by the scientific community as absolute truths when they are probably very far from the real incidence of this molecular finding.

Second, Southern blot methylation analysis using a U64 probe (encompassing p21 promoter from nucleotide [nt] – 571 to + 518) revealed the same results obtained after DNA digestion with HpaII followed by polymerase chain reaction (PCR). Moreover, normal bone marrow and peripheral blood showed incomplete methylation patterns, with bands corresponding to both methylated and unmethylated states. Similar results have been obtained analyzing peripheral lymphocytes by Chen et al.6 Therefore, it is surprising that Shen et al did not find p21 methylation when even healthy individuals show a partially methylated status. The p21 gene has a complex promoter and while some of its CpG sites are methylated, individuals show a partially methylated status. The p21 gene and premature. We wish to illustrate that significant mucocutaneous eruptions can be managed, enabling long-term use of this agent on clinical grounds. We recommend that Shen et al revise some aspects of their methodology and premature. We recommend that Shen et al revise some aspects of their technology and patient selection.

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References

To the editor:

Managing cutaneous reactions to imatinib therapy

Cutaneous reactions to imatinib therapy are increasingly being recognized, with up to 21% of patients experiencing mild to moderate reactions at doses of 600 mg and higher.1 A recent case report of Stevens-Johnson syndrome complicating the use of imatinib2 expressed caution in the widespread use of this agent based on such reactions. We wish to illustrate that significant mucocutaneous eruptions can be managed, enabling long-term continuation of imatinib therapy.

Case 1 involved a 66-year-old woman with chronic-phase chronic myeloid leukemia (CML) who was intolerant of therapy with interferon-α and was started on imatinib at a dose of 400 mg daily. After 10 days of therapy, she developed a macular, pruritic rash together with mild periocular edema. The following day, this had progressed into a disseminated rash with confluent areas over the torso and upper limbs. In addition, there were oral and vaginal mucosal erosions. The skin was not biopsied, but a clinical