CORRESPONDENCE

Microsatellite Instability in Myeloid Leukemias

To the Editor:

Somatic instability of variable number of tandem repeat (VNTR or microsatellite) sequences has been described recently in colorectal cancer as an indicator of a novel mechanism of carcinogenesis. The recent report in Blood by Wada et al suggested that microsatellite instability is a frequent feature in the evolution of chronic myeloid leukemia (CML) from chronic phase to blast crisis, and therefore, that defects in replication and/or repair mechanisms may be relevant to progression of CML. Our own observations provide no support for this conclusion.

We have studied 48 CML blast-crisis patients by comparing constitutional DNA extracted from buccal epithelial cells or chronic-phase leukocytes with DNA obtained from blast-crisis leukocytes. Twelve VNTR loci from six different chromosomes were amplified and, after autoradiography, the size as well as the intensity of amplified products were compared between constitutional and blast-crisis samples. Occasional instances of loss of heterozygosity (LOH) were found, but we did not find a single case of microsatellite instability, defined as the occurrence of novel alleles at more than one VNTR in a single sample. We have also compared buccal epithelial and blast cell DNA from 17 patients with acute myeloid leukemia of various French-American-British subtypes and similarly failed to find any convincing microsatellite instability (unpublished observations, June 1994). Out of a total of 649 paired amplifications, only 7 instances of novel VNTR bands in 6 acute leukemia samples were found. We believe these novel bands may simply reflect the naturally high mutation rate of VNTR sequences.

The reasons for the discrepancy between these two studies may be partly technical and partly interpretational. We have found that spurious PCR bands may be generated if the amplification conditions are suboptimal or if the DNA sample is impure. In this regard, it may be relevant that Wada et al used DNA extracted from Giemsa-stained slides. We believe that many of the instances of microsatellite instability described by Wada et al are unconvincing, especially for the primer pairs Mfd27 and Mfd41 shown in Figs 1 and 2 of their article. Data for the C13-9 and LPL primers was not shown, but microsatellite instability and LOH does appear to be clearer at the deleted in colorectal carcinoma gene (DCC) locus. However, we have also studied the DCC VNTR in 49 paired samples and found no instances of repeat instability or LOH in the 38 cases who were informative. Whether abnormalities in the DCC gene, or genes such as hMLH1 and hMSH2 that lead to microsatellite instability are relevant to CML or other leukemias will ultimately be determined by mutational and functional analysis.

We greatly appreciate the highly significant comments of N.C.P. Cross et al on genomic instability in the CML blast crisis. The letter suggests that the instability in VNTRs not to be associated with the 48 CML blast crisis. Their results on VNTR instability are valid and essential to the clarification of the mechanism of replication error (RER) in CNL, whereas LOH in CNL was not similarly affected, whereas LOH in VNTR could be detected in some cases. This finding is consistent with the previous observation that over 30 VNTR loci are not affected by RER phenotypes in colorectal and endometrial cancers.

In microsatellite repeats, instability may occur as a result of slippage during replication of repeat regions; the mismatch repair system is presumably affected differently from those of VNTR.

REFERENCES


Response

The data of N.C.P. Cross et al are at variance with our results in regard to microsatellite instability in BLOOD. DNA sample impurity or polymerase chain reaction (PCR) artifacts would not be the reason for this, but rather, differences in structure and replication/repair mechanisms of VNTRs and microsatellites. VNTRs and microsatellites are polymorphic DNA markers. Structurally, the former is comprised of tandem repeats and the latter, repeats of di, tri, or tetranucleotides. The replication/repair mechanisms of the repeats may possibly be different.

All RER' blast crises evaluated in our paper had been assessed for instability of three VNTR markers. Despite the frequent instability in dinucleotide repeats in the RER' blast crisis, VNTR was not similarly affected, whereas LOH in VNTR could be detected in some cases. This finding is consistent with the previous observation that over 30 VNTR loci are not affected by RER' phenotypes in colorectal and endometrial cancers. This, along with the data of N.C.P. Cross et al, suggests mismatch repair systems in microsatellites to possibly differ fundamentally from those of VNTR.
Microsatellite instability was detected in DNAs from fresh blast-crisis leukocytes. To rule out the possibility of PCR artifacts, RER+ blast crisis and fresh cases were analyzed using a 373 DNA sequencer (Applied Biosystems, Foster City, CA) with 672 Genescan-primer mix (Applied Biosystems, Foster City, CA) with 672 Genescan-primer mix. The human mutator genes, such as hMSH2 and hMLH1, have been cloned and direct sequencing of the mutator genes, such as hMSH2 and hMLH1, is now being conducted to investigate the fresh RER+ blast crisis. The mutational inactivation of these mutator genes is considered by us to uphold the conclusions specified in our report in BLOOD.

We should like to stress that the scrutiny and concern on the part of N.C.P. Cross et al are highly welcomed and appreciated. It is sincerely hoped that this letter in response will be found adequate to their points of inquiry.

NOTE ADDED IN PROOF


Chieki Wada
Department of Clinical Pathology
Kitasato University, School of Medicine,
Kanagawa, Japan

REFERENCES


4. Cleaver JE: It was a very good year for DNA repair. Cell 76:1, 1994


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NC Cross, H Silly and JM Goldman