Volume 14 Number 20 1986 Nucleic Acids Research

A variable tandem repeat locus mapped to chromosome band 10q26 is amplified and rearranged in leukocyte DNAs of two cancer patients

Mark Colb[1,2], Teresa Yang-Feng[3], Uta Francke[3], Brion Mermer[1,2], David R.Parkinson[1] and Theodore G.Krontiris[1,2]*

[1]Department of Medicine (Hematology/Oncology), Tufts-New England Medical Center, [2]Department of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA and [3]Department of Human Genetics, Yale University School of Medicine, New Haven, CT, USA

Received 3 July 1986; Revised 4 September 1986; Accepted 25 September 1986

ABSTRACT

A highly polymorphic locus associated with the variable tandem repetition of a 35 bp consensus sequence was mapped to chromosome 10, band q26. Examination of leukocyte DNA from a cancer patient revealed the twenty-fold amplification of one allelic fragment of this locus, while the other allelic fragment demonstrated a normal copy number. In another patient, Southern blotting of leukocyte DNA detected the deletion of the 3'-flanking region from one tandem repeat allele. These results indicate that variable tandem repeats may mark highly unstable regions of DNA in the human genome which can be altered by changes more extensive than simple tandem repeat variation.

INTRODUCTION

The tandem repetition of short (14-65 bp) nucleotide sequences is an important source of human genetic polymorphism. Variable tandem repeats (VTRs, minisatellites) generate allelism at the insulin (1), ζ-globin (2) and Ha-*rasl* (3) loci. A large family of related VTRs are distributed throughout the human genome and often show restriction fragment length variation (4,5). At the Ha-*rasl* locus, at least twenty-four distinct alleles have been detected; fifteen of these have thus far occurred only in cancer patients (6,7). Given the apparent instability of these tandemly-repetitive sequences and the unusual behavior of the Ha-*rasl* VTR, we have begun to identify and clone independent VTR loci for further studies comparing cancer patients and cancer-free controls. As reported here, one such locus, VTR4.1, was mapped to the long arm of chromosome 10 (band q26) by *in situ* hybridization and Southern blot analysis of somatic cell hybrid DNA. We have observed amplification of VTR4.1 in the leukocyte DNA of a patient with colorectal carcinoma and rearrangement, by deletion of 3' sequences flanking the VTR, in leukocyte DNA from a bladder cancer patient. These findings suggest that minisatellite regions can be associated with structural changes more extensive than simple tandem repeat variation. This is also the first demonstration of significant sequence amplification in constitutional DNA.

MATERIALS AND METHODS

Clone Isolation

Probe was prepared by nick-translation (8) of the MspI fragment containing the Ha-*rasl* tandem repeat (3), purified from the plasmid, pEC (9). A human genomic library (10) was screened by *in situ* plaque hybridization (11) at 50°C, in buffer containing 6X SET (1X SET = .15M NaCl, 1mM EDTA, 10mM Tris HCl, pH 7.5). Filters were washed twice at room temperature in 2X SET and twice at 37°C in 2X SET. One strong signal was obtained from 200,000 phage screened. Restriction mapping of the purified phage and Southern blotting (12) with the probe described above identified the region of homology to the Ha-*rasl* VTR (Figure 1A).

DNA Sequencing

The sequencing strategy was to subclone into the BamHI site of pBR322 a nested set of BamHI-BglII fragments spanning the VTR from the 5' BamHI site (Figure 1A). Five such subclones, containing increasing lengths of VTR (pBB10, pBB11, pBB119, pBB120 and pBB13), were then sequenced by the Maxam-Gilbert method (13). The sequence of both strands, proceeding toward the 5' BamHI site of Figure 1, was determined following 5' and 3' end-labelling at the pBR322 MspI site 12 bp from the 3' terminus of the VTR insert. The 3' flanking sequence was obtained directly from a clone bearing the VTR4.1 BamHI fragment by labelling at the terminal BglII site of the VTR and sequencing in the direction of the 3' BamHI site of Figure 1.

Chromosome Mapping

In situ chromosomal hybridization was carried out according to published procedures (14,15).

RESULTS

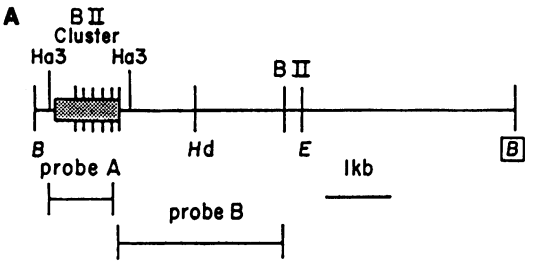
A human genomic library was screened at low stringency with purified Ha-*rasl* VTR as probe; one recombinant phage was isolated. The fragment of this phage homologous to the Ha-*rasl* VTR (Figure 1A) was purified and subcloned. It revealed considerable polymorphism in a panel of human leukocyte DNAs probed at high stringency (7) (see also below). When the DNA sequence of this region was obtained (Figure 1B), we observed a tandem array of 35 bp monomers, occasionally interspersed with 37-mers bearing a "TT" insertion. Many of the monomers contained a BglII site (AGATCT), as expected from restriction mapping which localized a BglII cluster to the Ha-*rasl*-homologous region of 4.1 (Figure 1A).

We previously characterized the VTR4.1 locus in a Southern blot survey of

HaeIII-digested leukocyte DNAs from 386 unrelated caucasians, of whom 226 were cancer patients (7). (HaeIII sites located 80 bp upstream and approximately 200 bp downstream most closely spanned VTR4.1, offering the greatest resolution of polymorphic fragments.) Probe A (Figure 1A), which contained only 80 bp of non-tandem repeat, was used. Seven fragments, a1-a7, were detected. All fragments but a2, which migrated too near a1, could also be resolved with MspI and PstI, ruling out multiple HaeIII site polymorphisms. Therefore, VTR4.1 represented an insertion/deletion polymorphism. Typical of VTR-based polymorphism (1,6), larger fragments of VTR4.1 demonstrated more intense hybridization, reflecting an increased number of tandemly-repetitive sequences binding the probe. For example, patient 1 (Figure 2A) possessed a1 and a5 fragments; the a5 hybridization intensity considerably exceeded that of the shorter a1 fragment. We also noted that VTR4.1 was linked to a BamHI site polymorphism 6 kb 3' to the tandem repeat (Figure 1A). For a1/a1 VTR homozygotes, the BamHI polymorphism yielded fragments of 7.2 and 8.7 kb (Figure 3A, lane 3).

Although DNA from patient 1, an individual with bladder cancer and an unremarkable family history, demonstrated the a1 and a5 fragments by HaeIII digestion (Figure 2A), BamHI digestion revealed a unique fragment of 5.4 kb with probe A (Figure 3A, lane 1). Because the intensity of hybridization of the 5.4 kb band exceeded that of the 8.7 kb band in the same DNA, we concluded that the shorter BamHI fragment contained the larger (a5) VTR. Furthermore, the larger BamHI fragment of patient 1 comigrated with the 8.7 kb BamHI fragment of the reference sample from an a1/a1 VTR homozygote (Figure 3A, lane 3).

To characterize the rearrangement of VTR4.1 in this patient, we rehybridized his BamHI-digested DNA with probe B. In this instance the flanking sequence probe barely recognized the 5.4 kb fragment (Figure 3B, lane 1. A longer exposure of this autoradiogram shows the faint 5.4 kb fragment more clearly). This indicated that in the aberrant band, the VTR sequences (represented by probe A) were no longer linked to the 3' flanking sequences (represented by probe B). The simplest explanation was that most, if not all, of the 2.4 kb flanking region had been deleted. Consistent with this interpretation, no new bands were detected by probe B in either BamHI (Figure 3B, lane 1) or BglII digests (Figure 3C, lane 1). Hence, rearrangement by translocation seemed less likely. Finally, the BglII digest of DNA from patient 1 demonstrated a 2.4 kb band of reduced intensity (Figure 3C, lane 1), suggesting haploidy for the 3' flanking region.



B

```
                                  .....aacaagaaag
 1   AGG GGGAGAC A CCACACT  CAA TGACCA A ATCTC
 2   AGGAGGGAGA T GCCACACT  CAACTGA A CA C ATCTC
 3   AGGA C GGAGACGCCACACT  CAAC AACTT A C ATC CA
 4   AGGAGGGAGACGCCACACTTTC C ACTGACCAGATCTC
 5   AGGAGGGAGAC A CCA T ACT  CAA TGACCAGATCTC
 6   AGGAGGGAGACGCCACACT  CAAC GACCAGATCTC
 7   AGGAGGGAGACGCCACACT  CAAC GACCAGATCTC
 8   AGGAGGGAGACGCCACACT  CAAC GACCAGATCTC
 9   AGGAGGGAGACGCCACACTTTC C ACTGACCA....
10-12.....[3 repeats].............CCAGATCTC
13   AGGAGGGAGACGCCACACTTTCAACTGACCAGATCTC
14   AG A AGGGAGACGCCACACT  CAAC GACCAGATCTC
15   AGGAGGGAGACGCCACACT  CAACTGACCAGA C CTC
16   AGGAGGGAGACGCCACACT  CAACTGACCAGATCTC
17   AGGAGGGAGACGCCACACT  CAACTGACCAGA C CTC
18   AGGAGGGAGACGCCACACT  CAACTGACCAGA C CTC
19   AGGAGGGAGACGCCACACT  CAACTGACCAGA C CTC
20   AGGAGGGAGACGCCACACT  CAACTGACCAGATCTC
21   AGGAGGGAGACGCCACACT  CAA TGACCAGATCTC
22   AGGA C GGAGACGCCACACTTTCAACTGACCAGATCTC
23   AGGAGGGAGA T GCCACACT  CAACTGACCAGA C CTC
24   AGGAGGGAGACGCCACACT  CAACTGACCAGA C CTC
25   AGGAGGGAGACGCCACA.................
26-27.............[1-2 repeats].......ATA C
28   AGGAGGGAGAC A CCACA TT CA T Caccgtctgatga
```

Figure 1: Restriction Map (A) and Nucleotide Sequence (B) of VTR4.1. (A) The stippled box marks the region related to the Ha-rasl VTR. The vertical lines within the box represent multiple BglII sites. Probe A is the 0.9 kb HaeIII fragment of pBB119 (see below), purified by subcloning in pUC9. Probe B was the 2.4 kb BglII fragment purified from the depicted BamHI fragment by low-melting-point agarose gel electrophoresis (16). B = BamHI; BII = BglII; Ha3 = HaeIII; Hd = HindIII; E = EcoRI. The boxed BamHI site represents a site polymorphism. (B) The 35 bp repeat is evident, with interspersed monomers containing an additional "TT". Sequence alignment from

overlapping clones was facilitated by the frequent occurrences of single-base departures from the consensus sequence. Three repeats in the middle of the VTR were not sequenced. The number of monomers in that portion of the VTR was determined by counting repeat units in the sequencing ladder from several independent subclones. Lower case letters represent the sequence of DNA flanking the VTR. A portion of the VTR4.1 35 bp consensus, GGAGACGCCACACTC, was a 14/15 nucleotide match with the corresponding region in the 28 bp consensus sequence of the Ha-rasl VTR. The two VTR sequences were otherwise entirely divergent.

Another sample from the population survey was that of a patient with colorectal cancer; his HaeIII-digested DNA demonstrated a very intense a1 band (Figure 2B). The patient, now deceased, had a father with testicular cancer, a sister with "brain" cancer and a brother with carcinoma of the jaw. He had received three doses of 5-fluorouracil, without depression of his leukocyte count. Lanes 2 and 3 of Figure 3A show, respectively, the DNAs of the patient and a reference sample, both heterozygous for the BamHI site polymorphism. The upper band in each lane was of normal intensity and served as an internal control for the amount of DNA blotted. The patient's highly amplified 7.2 kb

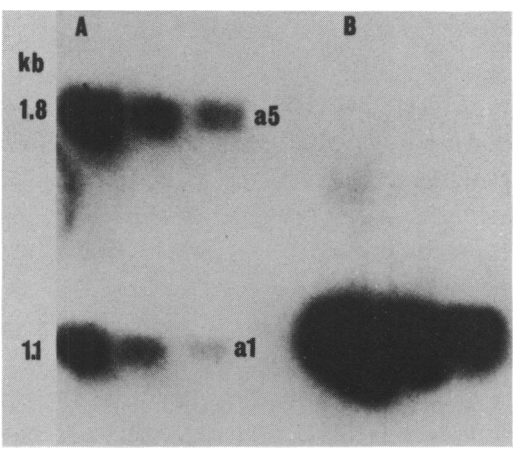


Figure 2: VTR4.1 Fragments in Two Cancer Patients. DNA was prepared from peripheral blood leukocytes as previously described (6). 5, 2.5 and 1.25 mcg of HaeIII-digested DNA of patient 1 (panel A) and patient 2 (panel B) were subjected to Southern blotting. Hybridization was to probe A (Figure 1A) in 6X SET at 65°C. Successive washes at the same temperature were in 2X, 1X, 0.5X and 0.1X SET. Fragments a1 (1150 bp) and a5 (1750 bp) were present in DNA of patient no. 1. The amplified fragment of patient no. 2 corresponded to an a1 fragment. Markers were HindIII-digested lambda and HincII-digested phiX174 DNAs.

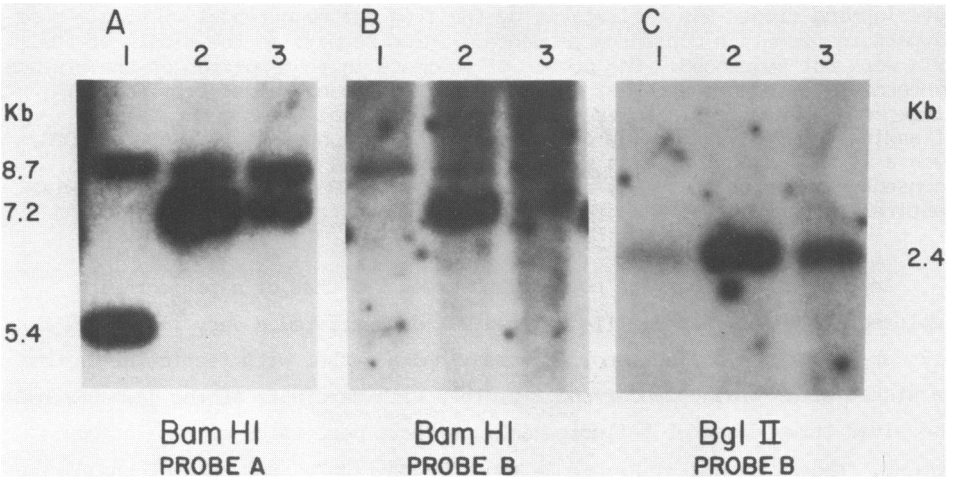


Figure 3: Rearrangement and Amplification of VTR4.1 in Two Cancer Patient DNAs. Five mcg. of DNA from patient no. 1 (lane 1), patient no. 2 (lane 2) and a reference individual homozygous for the VTR4.1 a1 allele (lane 3) were digested with BamHI (panels A and B) and BglII (Panel C). The resulting Southern blots were hybridized under the same conditions as Figure 2 to probe A (panel A) or probe B (panels B and C). Panel B is the rehybridization of filter in panel A with probe B. Marker was HindIII-digested lambda DNA.

fragment was clearly evident. By determining the difference in film exposure time required to equalize the 7.2 kb and 8.7 kb band intensities, we estimated that 20-fold amplification had occurred in this patient.

Since the normal 7.2 kb BamHI fragment was apparently released from the amplified region (Figure 3A, lane 2), the borders of the amplicon were outside the BamHI sites. Therefore, sequences distinct from the VTR region would be coamplified. To confirm this prediction, we hybridized probe B (Figure 1A) to the same BamHI-digested DNA (Figure 3B). This 2.4 kb probe, representing the 3' flanking sequences, contained only 25 bp from the VTR region and did not recognize, under our hybridization conditions, the same VTR-specific HaeIII bands present in Figure 2 (data not shown). The probe B sequences were represented in the amplicon (Figure 3B, lane 2). This did not result from incomplete erasure of probe A: note the reversal of band intensities in 3A, lane 1 vs. 3B, lane 1. Further corroboration was obtained in the BglII digestion of these DNAs (Figure 3C). The 2.4 kb fragment corresponding to probe B was more intense in patient 2 than in the control.

We had chromosomally mapped VTR4.1 prior to our population survey. In

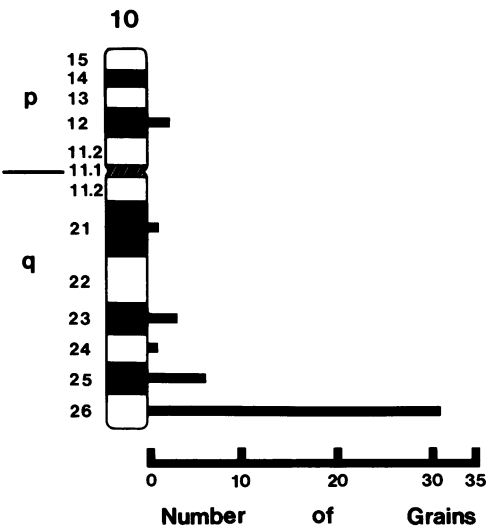


Figure 4: Chromosomal Location of VTR4.1. Silver grain distribution along chromosome 10 (ideogram from ISCN 1985, ref. 17). Probe A was nick-translated with [^{3}H]dATP, [^{3}H]dCTP and [^{3}H]dTTP to a specific activity of 2.3 X 10^7 cpm/mcg.

situ hybridization of ^{3}H-labeled probe A to human chromosome preparations resulted in specific labeling at band q26 of chromosome 10. Of 178 silver grains on 100 metaphase spreads, 31 (17.4%) were found at 10q26 (Figure 4); no other chromosomal site was labeled above background. Southern blot analysis of DNA prepared from 15 human X Chinese hamster somatic cell hybrids has independently confirmed this chromosomal assignment. A single 14 kb EcoRI fragment was detected in human control DNA and in hybrids containing human chromosome 10 (data not shown). The presence of the 14 kb band was completely concordant with the presence of human chromosome 10. All other chromosomes were excluded by two or more discordant hybrids. VTR4.1 was unlinked to other, previously characterized, VTRs. Furthermore, it did not map within regions known to undergo rearrangement during differentiation or amplification during tumor progression.

For patient 1, 24-hr non-stimulated, and pokeweed- and phytohemagglutinin (PHA)-stimulated, methotrexate-synchronized peripheral blood cultures were processed for chromosome preparations. No metaphase spreads were found in 24-hr, non-stimulated cultures, indicating that there were probably no spontaneously-dividing malignant cells among the leukocytes of patient 1.

Chromosome analysis of 13 cells from pokeweed- and PHA-stimulated cultures revealed a normal karyotype. No cytological rearrangement of chromosome 10 was observed at the 850-band stage when an additional 40 pairs of chromosome 10 were examined. We were unable to perform cytogenetic analysis for patient 2.

DISCUSSION

The amplification of drug resistance markers and oncogenes has been detected in both cell lines and tumor tissue. DNA deletions have been observed in structural gene loci, most often associated with some disease phenotype. We have detected these types of large-scale structural changes involving a VTR locus in the leukocyte DNA of two hematologically normal cancer patients. Therefore, it is unlikely that these changes originated in tumor cell genomes. The alterations were either inherited by the affected patients or arose early in embryogenesis. We cannot say whether the new DNA configurations were transmissible to progeny because family members were unavailable for study. It will be important to determine (1) if such structural instability is limited to VTR4.1 or is present in other, similar regions of the human genome; and (2) if this instability is related to cancer predisposition. Conceivably, constitutional factors that operate to increase cancer risk might also leave their mark upon germline VTRs.

ACKNOWLEDGEMENTS

We thank Kathleen Barrett for a critical reading of the manuscript. Joyce Lonergan provided excellent technical assistance. This work was supported by grants from the National Institutes of Health (CA37866 to T.G.K. and GM26105 to U.F.). M.C. received support from postdoctoral training grant, CA09429, and T.Y.-F. from GM07439. T.G.K. was a fellow of the John A. and George L. Hartford Foundation.

*To whom correspondence and reprint requests should be addressed: Division of Hematology/Oncology, New England Medical Center, Box 245, 750 Washington St., Boston MA 02111, USA

REFERENCES

1. Bell GI, Selby MJ and Rutter WJ. (1982) Nature 295:31-35.
2. Goodbourn SEY, Higgs, DR, Clegg JB and Weatherall DJ. (1983) Proc Natl Acad Sci USA 80:5022-5026.
3. Capon DJ, Chen EY, Levinson AD, Seeburg PH and Goedell DV. (1983) Nature 302:33-37.

4. Jeffreys AJ, Wilson V and Thein SL. (1985) Nature 314:67-73.
5. Jeffreys AJ, Wilson V and Thein SL. (1985) Nature 316:76-79.
6. Krontiris TG, DiMartino NA, Colb M and Parkinson DR. (1985) Nature 313:369-374.
7. Krontiris TG, DiMartino NA, Colb M, Mitcheson HD and Parkinson DR. (1986) J Cell Biochem 30:319-329.
8. Maniatis T, Jeffreys A and Kleid DG. (1975) Proc Natl Acad Sci USA 72:1184-1188.
9. Chang EH, Furth ME, Scolnick, EM and Lowy DR. (1982) Nature 297:479-483.
10. Lawn RM, Fritsch EF, Parker RC, Blake G and Maniatis T. (1978) Cell 15:1157-1174.
11. Maniatis T, Fritsch EF and Sambrook J.(1982) Molecular Cloning, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. Southern EM. (1975) J Mol Biol 98:503-517.
13. Maxam A and Gilbert W. (1980) Meth Enzymol 65:499-560.
14. Harper ME and Saunders GF. (1981) Chromosoma 83:431-439.
15. Yang-Feng TL, Seeburg PH and Francke U. (1986) Somat Cell Mol Genet 12:95-100.
16. Schmitt JJ and Cohen BN. (1983) Anal Biochem 133:462-464.
17. ISCN 1985. (1985) An International System for Human Cytogenetic Nomenclature. S. Karger, Basel.