Analysis of somatic mutations at short tandem repeat loci in colorectal carcinomas

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INTRODUCTION

To further analyse the variability at tandem repetitive loci, we have studied somatic mutation events in a subset of four short tandem repeats (STRs or microsatellites). Pairs of human blood leukocyte and colorectal adenocarcinoma DNA were adopted as a somatic model system.

The existence of new tumour alleles in colorectal (Lothe et al. 1993; Thibodeau et al. 1993; Hoff-Olsen et al. 1995) and other tumours (Han et al. 1993; Risinger et al. 1993; Orth et al. 1994) have been extensively described. Most reports focus on dinucleotides and the mere registration of new tumour alleles. The somatic instability recorded is called replication error (Aaltonen et al. 1993), reflecting the mutation mechanism. With focus on the evolution, maintenance and variability of repetitive loci, the nature of mutational events becomes an important issue.

Several reports addressing the mutational mechanism responsible for the occurrence of new microsatellite alleles, conclude that the most likely definitive event is intra-chromosomal, i.e. strand slippage or sister chromatide exchange. These reports are based mainly on either studies of flanking sequences (Morrall et al. 1991; Mahtani et al. 1993) or evaluation of the fitness of the observed mutations to a certain mutation model (Pena et al. 1993; Shriver et al.1993).

Microsatellite mutations in CEPH pedigrees show an almost 2:1 ratio of gains over losses of alleles (Weber and Wong 1993). Rubinsztein et al. (1995) most recently pointed to the fact that microsatellite allele length distributions more often show positive than negative skews, consistent with a bias toward gains over losses. There is also evidence that *mini*satellites display a tendency toward allelic expansion vs. contraction at mutation (Jeffreys et al. 1988; Olaisen et al. 1993). The instability of trinucleotide repeats associated with several heritable diseases is also associated with a bias toward expansion at mutation (Kuhl and Caskey 1993).

We here report data on the nature and distribution of new tumour alleles at the four tetranucleotide STRs HUMTHO1, HUMFES/FPS, HUMVWA31/A and HUMF13A1.

MATERIALS AND METHODS

DNA was extracted from blood and carcinoma of 217 unselected patients. Meling et al. (1991) have presented the carcinoma material. Polymerase chain reaction (PCR) with fluorescent dye-labelled primers (Kimpton et al.1993), electrophoresis and analysis were performed using a 373A DNA Sequencer (Applied Biosystems,Inc.) as described (Hoff-Olsen et al. 1995).

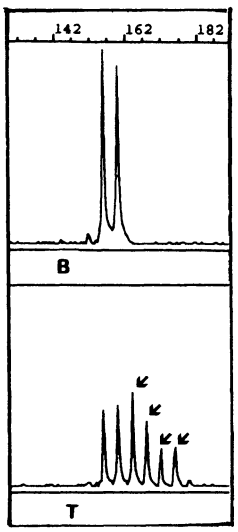
RESULTS

Mutations are detected with all four markers . The somatic mutation frequencies range from 0.009 (HUMF13A1) to 0.122 (HUMVWA31/A). In the four STRs as a group, there is a significant excess of allelic expansion vs. contraction at mutation (43 vs. 24 tumours).

The mutant alleles are consistently composed of perfect integers of the four base pair repeats. Electrophoretogram reading confirmed this by locating the mutated alleles 4, 8, 12, or 16 base pairs to either side of the assigned constitutional allele (fig. 1). Furthermore, the new tumour bands could all be assigned to known alleles in the parent (Norwegian) population (Myhre Dupuy et al. 1993).

46 of the 61 tumours (75%) with mutant bands display only a single new band which differ with one (74%), two (15%) or three (11%) repeats from the constitutional band. The remaining 15 samples (25%) show either biallelic events (53%) or multiple consecutive mutant bands ("smears") (47%) (fig 1).

Figure 1



Electrophoretogram image.

Periferal blood (B) and colorectal carcinoma (T) DNA amplified with microsatellite marker HUMVWA31/A displaying "smear" of expanded new tumour alleles.

New alleles indicated (arrowheads).

Scale, X-axis: Basepairs (allele sizes).

DISCUSSION

Several reports conclude that human polymorphic tandem repeats tend to expand vs. contract at both somatic and germline mutation (Jeffreys et al. 1988; Olaisen et al. 1993; Weber and Wong 1993). The present study of somatic STR mutations strongly supports this observation.

Wooster et al (1994) found that alleles with greater number of repeats seem more likely to exhibit instability in tumours, supporting the suggestion that new mutants in triplet diseases originate from population subgroups with the longest "normal" repeats (Richards and Sutherland 1994). In contrast, in the four STR`s of this study we observe that the assigned constitutional alleles of the mutant tumour alleles are nearly evenly distributed over the allele frequency distributions of the parent population (data not shown).

Others have shown that colorectal adenomas display single mutant bands at mutation, while carcinomas tend to show smears (Lothe et al, 1993; Lothe RA, pers. comm.). Our present results also indicate that there are at least two such different types of somatic mutations. We will perform further analyses to clarify any eventual clinical significance of this observation.

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