SOMATIC INSTABILITY IN CANCER AT SEVEN TETRAMERIC STR LOCI USED IN FORENSIC GENETICS

A. Alonso, P. Martín*, C. Albarrán*, A. Guzman***, B. Aguilera**, H. Oliva*** and M. Sancho**

* Sección de Biología ** Sección de Anatomía Patológica. Instituto de Toxicología. Mº de Justicia e Interior. Madrid. SPAIN.
*** Fundación Jimenez Díaz. Departamento de Anatomía Patológica.Madrid. SPAIN.

INTRODUCTION

Microsatellite repeats have been reported to be unstable in some inhereted diseases (Caskey et al. 1992; Richards et al. 1992) and in several human cancers (Aaltonen et al. 1993; Thibodeau et al. 1993; Ionov et al. 1993). Abnormalities in the DNA mismatch-repair pathway have been proposed as being responsible for microsatellite instability in some type of cancers (Fishel et al. 1993; Leach et al. 1993).

In an attemp to evaluate the impact that the microsatellite instability observed in human cancers could have in some forensic DNA studies (i.e. genetic identification of fixed tumor specimens that were thought to have been mis-paired, paternity testing from fixed tumor biopsies in cases involving deceased parents,...) we have analyzed seven polymorphic STR loci (TH01, TPOX, CSF1PO, VWA, FES/FPS, F13A1, and F13B) in DNAs extracted from paired normal and colorectal or gastric tumor tissue samples corresponding to 21 individuals.

Our preliminary results show a high incidence of allele gain in gastric tumors that affects multiple STR loci concurrently, and a relatively low incidence of genetic instability in colorectal tumors restricted to allelic imbalance at the CSF1PO locus.

MATERIALS AND METHODS

The DNA was extracted by the standard phenol/chloroform extraction procedure from fresh-frozen tumor (13 colorectal adenocarcinomas and 8 gastric adenocarcinomas) and normal tissues corresponding to 21 individuals. The amplification of STR loci was performed by single-locus PCR reactions in the case of VWA, FES/FPS, F13A1, and F13B loci or by a multiplex PCR reaction in the case of TH01, TPOX, and CSF1PO loci according to the manufacturer's recommendations using the GenePrint STR System (Promega Corporation, Madison, WI, USA). PCR products were analyzed by denaturing polyacrylamide gel electrophoresis and subsequent detection by silver stain (Budowle et al. 1991; Martín et al. 1995). Alleles were designed according to the number of the repeat units using sequenced allelic ladders. Samples that showed differencies in the STR profiles between tumor and normal DNAs were reanalyzed from a second DNA extract.

RESULTS AND DISCUSSION

Instability at one or more loci was observed in 75% (6/8) of gastric tumors and 15% (2/13) of colorectal tumors (Table 1).

Instabilities were apparent for the majority of gastric tumors as partial allelic losses as well as extra-alleles of different size in the tumor that were not present in the normal DNA (Fig. 1). 17 out of 21 STR instabilities observed in gastric tumors were extra-alleles, while only 4 were partial allelic losses. The difference in size between the extrabands observed in the tumors and the closest allele detected in the paired normal DNAs varied from one to six repeat units (Fig. 1). The only instability observed in 2 out of 13 colorectal tumors analyzed was a partial allelic loss at the CSF1PO locus (chromosome 5p). The loci that presented higher numbers of instabilities were CSF1PO (4 cases of allele imbalance and 3 cases of extra-alleles out of 21 cases) and VWA (5 cases of extra-alleles out of 21 cases).

Our results support previous studies which showed a high incidence of STR instability in gastric tumors (Han 1993) and also allow to confirm two patterns of genetic instability in sporadic cancers: allele gain which is the most frequent pattern of instability observed in the present study in gastric adenocarcinoma and allele loss which is the only STR instability (restricted to the CSF1PO locus) observed in colorrectal adenocarcinoma. Apart from cancer type, other factors that could influence the pattern of instability (tumor stage, sequence of the STR repeat unit, chromosomal location,...) should be further investigated.

In conclusion, although the incidence of instability in tetrameric STR loci depends on the type of cancer and other factors, this kind of genetic alterations should be taken into account as a potencial source of error when interpreting STR profiles obtained from neoplasic tissues in identity testing studies.

Table 1. Abnormalities of microsatellite repeats found in tumors.

MICROSATELLITE MARKER			TUMOR TYPE				TOTAL MARKER
Chromosome Location	Name	Repeat unit sequence	Colon		Stomach		
			Pattern of Instability				
			Loss	Gain	Loss	Gain	
1q31-q32.1	HUMF13B	AAAT	–	–	–	2	2 (9.5%)
2p13	HUMTPOX	AATG	–	–	–	2	2 (9.5%)
5q33	HUMCSF1PO	AGAT	2	–	2	3	7 (33.3%)
6p24-6p25	HUMF13A1	AAAG	–	–	1	1	2 (9.5%)
11p15.5	HUMTH01	AATG	–	–	1	1	2 (9.5%)
12p12-pter	HUMVWA	AGAT	–	–	–	5	5 (23.8%)
15q25-qter	HUMFES/FPS	AAAT	–	–	–	3	3 (14.3%)
TOTAL (tumor type)			2 (15.4%)		6 * (75%)		

(*) Total number of gastric tumors showing instabilities (three gastric tumors showed instability for 5 STR loci and the other three showed instability for two STR loci.

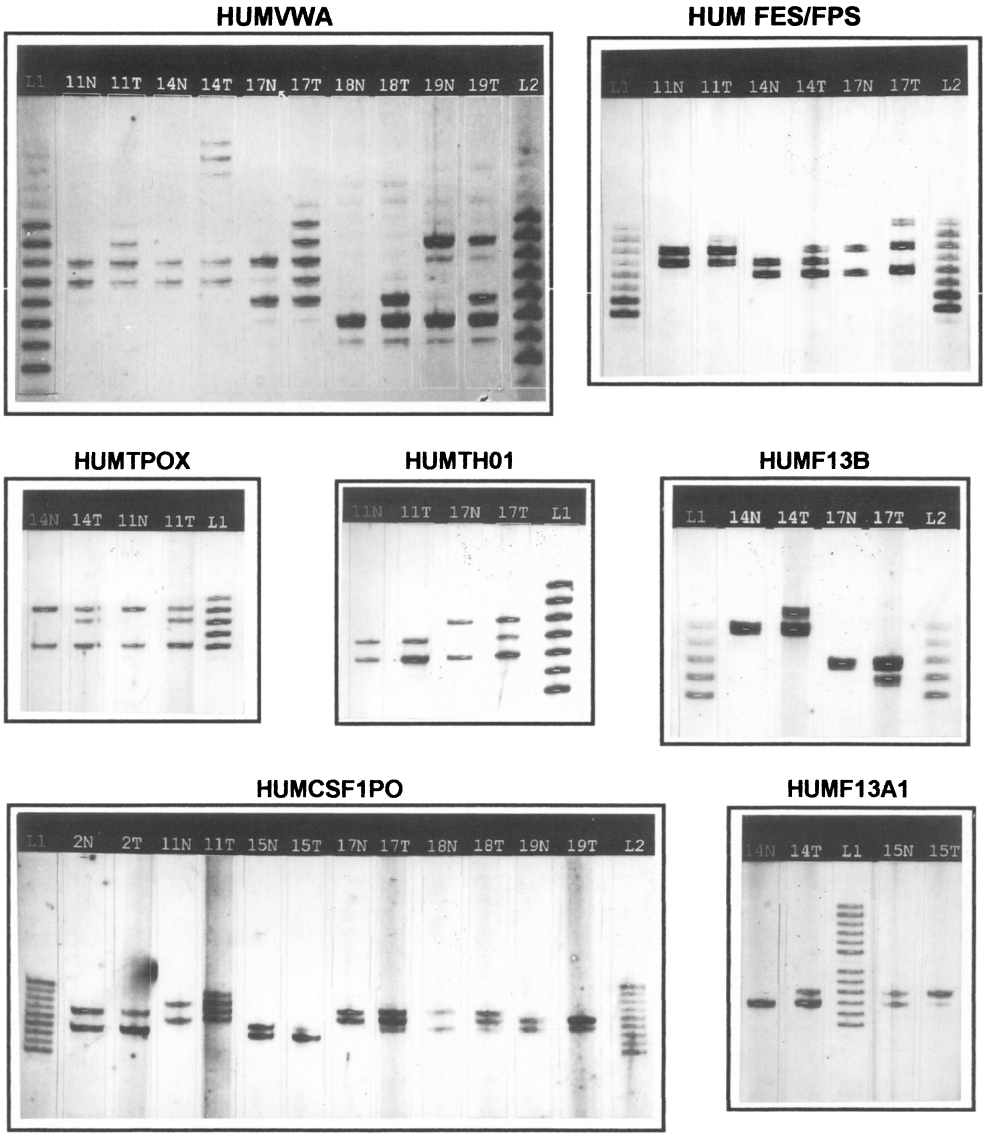

Fig. 1. Abnormalities of STR profiles in tumor DNA compared to constitucional STR profiles in normal DNA from the same patient. (L): allelic ladder.

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