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Infrequent microsatellite instability in oesophageal cancers

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Summary Alterations of microsatellites have been found at relatively high frequency in hereditary and sporadic colorectal cancer and gastric and pancreatic cancers and at lower frequency in some other cancers. We determined the frequency of instability at 39 poly-CA microsatellite loci in 20 squamous cell carcinomas and 26 Barrett's adenocarcinomas of the oesophagus. None of the tumours presented instability for a high percentage of the tested loci. Four squamous cell carcinomas and six Barrett's adenocarcinomas showed microsatellite instability at one locus, and three Barrett's adenocarcinomas showed microsatellite instability at two loci. The presence of few loci showing microsatellite instability could be due to an instability background. We conclude that genetic defects in the DNA mismatch repair system do not play an important role in oesophageal cancers.

Keywords: microsatellite instability; cancer of the oesophagus; squamous cell carcinoma of the oesophagus; Barrett's adenocarcinoma; DNA mismatch repair system

Recently a new class of genetic alterations in human tumours has been described (Aaltonen et al, 1993; Thibodeau et al, 1993). These appear at microsatellite loci that are short, repeated nucleotide sequences distributed within the normal genome. Alterations of microsatellites consist of the loss or gain of one or more repeat units in tumours compared with matched normal DNA and have been termed microsatellite instability (MI). MI was first described in colorectal cancer, in both hereditary non-polyposis colorectal cancer (HNPCC) and sporadic colorectal cancer cases (Aaltonen et al, 1993; Thibodeau et al, 1993), as a result of a deficient DNA mismatch repair system (Fishel et al, 1993; Leach et al, 1993; Parsons et al, 1993; Bronner et al, 1994; Nicolaides et al, 1994; Papadopoulos et al, 1994). MI has also been observed in a variety of sporadic cancers, such as endometrium, stomach, kidney, ovary and pancreas cancers, belonging to the HNPCC tumour spectrum (Han et al, 1993; Peltomäki et al, 1993; Risinger et al, 1993). In bladder, breast and lung cancers, MI has been reported with variable frequency (Gonzalez-Zulueta et al, 1993; Merlo et al, 1994; Schridar et al, 1994). There are conflicting reports concerning the presence of MI in oesophageal cancers (Meltzer et al, 1994; Keller et al, 1995; Mironov et al, 1995; Nakashima et al, 1995; Gleeson et al, 1996). Cancer of the oesophagus is among the most common and severe malignant neoplasms in the world (Muller et al, 1990; Parkin et al, 1993). There are two main histological types of oesophageal cancer, squamous cell carcinoma (SCC) and Barrett's adenocarcinoma (BA). SCC is more frequent and is associated with alcohol and tobacco consumption in Western countries (Tomatis et al, 1990). BA develops in Barrett's oesophagus (Spechler and Goyal, 1986), an acquired metaplastic process resulting from chronic gastro-oesophageal reflux (Potet and Duchatelle, 1990). The mechanisms of carcinogenesis of the oesophageal mucosa are not entirely established.

In this study, we compared the incidence of MI in the two main types of oesophageal cancer and compared our results with those obtained in other series.

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MATERIALS AND METHODS

Tumours and DNA

Fresh resected specimens were collected during surgery at Beaujon Hospital (Clichy, France) from 1988 to 1994. Twenty SCCs and 26 BAs were included in the study. The patients had received neither radiation therapy nor chemotherapy before surgery. In all cases part of the tumour and part of the normal gastric mucosa were snap frozen and stored at –80°C until use. The surgical specimen was embedded in paraffin for histopathological analysis. The group of patients with SCC demonstrated a male–female ratio of 17:3 and a median age at diagnosis of 58 years (range, 44–72 years). In the patients with BA, the male–female ratio was 24:2 and the median age at diagnosis was 65 years (range 42–80 years). Consumption of alcohol and tobacco was known for 18 patients with SCC and 24 patients with BA; 15 patients with SCC (83%) and four with BA (17%) were chronic alcoholics; 14 patients with SCC (78%) and 15 with BA (58%) were smokers.

Genomic DNA was extracted from primary tumours and adjacent normal gastric mucosa by proteinase K digestion and phenol–chloroform extraction as described previously (Sambrook et al, 1989). p53 alterations had been searched previously in all tumours studied (Muzeau et al, 1996).

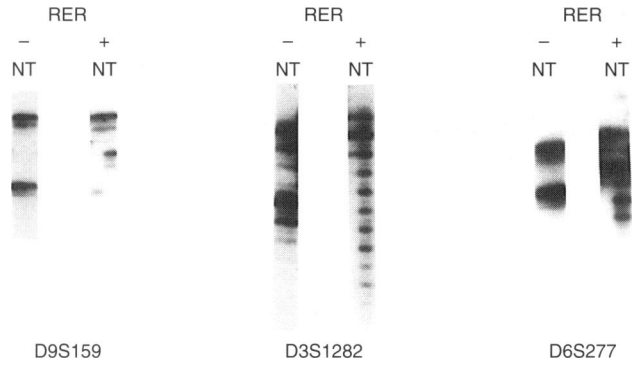

Figure 1 Examples of microsatellite instability in oesophageal cancers at different loci. N, normal DNA; T, tumour DNA

Table 1 Main clinical and morphological features of oesophageal carcinomas included in the study

	Squamous cell carcinoma (*n* = 20)		Barrett's adenocarcinoma (*n* = 26)	
	With no MI (*n* = 16)	With MI at one or two loci (*n* = 4)	With no MI (*n* = 17)	With MI at one or two loci (*n* = 9)
Mean age (years)	58	59	65	64
Male–female	3:13	0:4	1:16	1:8
Alcoholic	12 (14[a])	3(4[a])	4 (16[a])	0 (8[a])
Smokers	11 (14[a])	3(4[a])	10 (16[a])	4 (8[a])
p53+[b]	15	3	16	8
UICC grade				
I			6	3
IIa	5		4	2
IIb	2	1		
III	9	3	6	4
IV			1	

MI, microsatellite instability. Alcoholic, consumption of alcohol > 80 g per day; smokers, consumption of tobacco > 20 packet–years. [a]Patients for whom alcoholic and smoking habits are known. [b]p53+, Mutation of p53 gene and/or accumulation of p53 protein (Muzeau et al, 1996).

Microsatellite instability

A total of 39 poly-CA microsatellite loci selected from among the published list from Genethon (Gyapay et al, 1994) were analysed. Four were located on chromosome 1 (D1S225, D1S229, D1S239, D1S306), two on chromosome 3 (D3S1282, D3S1297), one on chromosome 4 (D4S414), two on chromosome 5 (D5S393, D5S430), three on chromosome 6 (D6S309, D6S271, D6S277), three on chromosome 8 (D8S272, D8S277, D8S283), 14 on chromosome 9 (D9S152, D9S153, D9S156, D9S157, D9S158, D9S159, D9S161, D9S165, D9S168, D9S169, D9S171, D9S175, D9S197, D9S259), two on chromosome 10 (D10S199, D10S226), one on chromosome 13 (D13S175), one on chromosome 14 (D14S250), one on chromosome 15 (D15S128), one on chromosome 16 (D16S517), two on chromosome 17 (D17S784, D17S790), one on chromosome 18 (D18S53) and one on chromosome 20 (D20S107).

Primers specific for each locus were used to amplify the repeat and short flanking sequences from template DNA using multiplex polymerase chain reaction (PCR). Amplification was carried out in a 9600 Perkin Elmer Cetus thermal cycler, using an AmpliTaq kit (Perkin Elmer Cetus, Emeryville, CA, USA) in a final volume of 20 µl containing 25 ng of genomic DNA, 0.25 U of Taq polymerase, 0.3 µmol l^{-1} of each primer, 20 µmol l^{-1} of each dNTP, 1.5 mmol l^{-1} magnesium chloride and 1 × buffer. PCR was performed for 35 cycles, each comprising 30 s at 94°C, 30 s at 55°C and 60 s at 72°C. Aliquots of amplified DNA were electrophoresed on a 6% polyacrylamide, 32% formamide, 7 mol l^{-1} urea denaturing gel and transferred onto Hybond nylon membranes. Filters were hybridized with a ^{32}P-labelled $(CA)_{12}$ probe and autoradiographed.

RESULTS AND DISCUSSION

In this study, none of the tumours showed a high MI index. Four of 20 SCCs showed MI at one locus (2.5% of 39 loci tested). Among 26 BAs, six and three tumours showed MI at one (2.5% of loci tested) and two loci (5% of loci tested) respectively. Representative examples of MI are shown in Figure 1. The instability affected different loci on chromosome 3, 4, 6, 9, 17 and 18. No MI was demonstrated in any of the remaining cases.

Table 2 Microsatellite instability in oesophageal cancers

Authors	No. of tumours tested	No. of loci tested	No. of tumours with MI at *x* % loci tested		
			≤ 10%	10% < MI ≤ 40%	> 40%
Meltzer et al (1994)	36 BA	5	0	6	2
(USA)	42 SCC		0	1	0
Keller et al (1995)	15 BA	8	0	2	0
(Germany)					
Mironov et al (1995)	18 SCC	17	2	0	0
(France)					
Nakashima et al (1995)	29 SCC	5	0	4	2
(Japan)					
Gleeson et al (1996)	17 BA	139	16	0	1
(Northern Ireland)					
Present series	26 BA	39	8	0	0
(France)	20 SCC		4	0	0
Total	94 BA		24 (25%)	8 (8%)	3 (3%)
	109 SCC		6 (6%)	5 (5%)	2 (2%)

MI, microsatellite instability; BA, Barrett's adenocarcinoma; SCC, squamous cell carcinoma.

The characteristics of the 13 (28%) tumours that showed MI at one or two microsatellite loci compared with tumours without MI are presented in Table 1.

An association between the presence of MI and certain clinicopathological features has been reported in sporadic colorectal cancer (Lothe et al, 1993; Kim et al, 1994) and gastric cancer (Dos Santos et al, 1996). However, for both types of tumours, only those carcinomas with multiple replication error positive (RER+) loci were significantly associated with poor differentiation, rare nodal metastases and prolonged survival, whereas carcinomas with one or two loci instable were similar to RER- carcinomas (Lothe et al, 1993; Dos Santos et al, 1996). In our study, we also observed no differences between tumours with MI at one or two loci and those without instability, regarding their clinicopathological characteristics (Table 1).

Moreover, in colon cancer, tumours with instability at multiple loci were frequently diploid (Aaltonen et al, 1993). Although we did not study the DNA ploidy in our tumours, it has been reported that most oesophageal cancers have a DNA-aneuploid pattern (Robaszkiewicz et al, 1991; Nakamura et al, 1994), a feature that again suggests that those tumours do not behave as RER+ carcinomas. Altogether, it appears that our finding of MI in a single or in two loci does not imply a mutator phenotype for those tumours and that multiple alterations at microsatellite loci as described in RER+ cancers is very unusual in both types of oesophageal carcinomas.

We have found five series reporting MI in oesophageal cancer in the literature (Table 2). Together with our study, they included a total of 109 SCCs and 94 BAs (Meltzer et al, 1994; Keller et al, 1995; Mironov et al, 1995; Nakashima et al, 1995; Gleeson et al, 1996). Only the study by Meltzer et al (1994) and our series included both types of oesophageal cancer: SCC and BA.

Criteria for identifying RER+ tumours have not been precisely defined. Hamelin et al (1994*a*), analysing a series of colon cancers with more than 100 poly-CA microsatellite loci, found two types of tumours. A first group of tumours located predominantly in the right colon showed MI at more than 50% of loci tested and were considered as RER+. A second group showed MI between 0% and 10% of loci tested and were considered as RER-. It could be hypothesized that the presence of rare loci showing MI is explained by the existence of a background of instability (<10%), independent of genetic defects in the mismatch repair system. Therefore, it is necessary to examine numerous loci to categorize a tumour as RER+.

It is noteworthy from Table 2 that oesophageal tumours with an instability index between 10% and 40% were detected only in series analysed with a small number of microsatellites (Meltzer et al, 1994; Keller et al, 1995; Nakashima et al, 1995) whereas, in series analysed with at least 17 microsatellites (Mironov et al, 1995; Gleeson et al, 1996; present series), tumours had an instability index either below 10% or above 40%. This observation suggests that there is also a background of MI in oesophageal cancers and that only 3 out of 94 (3%) BA and 2 out of 109 (2%) SCC are really RER+ tumours in the literature.

A number of genetic changes have been demonstrated in both types of oesophageal cancer, including loss of heterozygosity involving the loci for several tumour-suppressor genes, such as Rb, p53, APC and DCC (Huang et al, 1992; Boyton et al, 1993). Mutation of the p53 tumour-suppressor gene has been reported as extremely frequent in BA (Hamelin et al, 1994*b*; Gleeson et al, 1995) and SCC (Audrezet et al, 1993; Muzeau et al, 1996). In colorectal cancer, it appears that there is an inverse relationship between the presence of p53 gene mutation and the RER+ phenotype (Kim et al, 1994; Cottu et al, 1996). If the same phenomenon occurs in oesophageal carcinogenesis, a high percentage of p53 mutation may make it unlikely to find tumours that are characteristic of the RER+ phenotype.

We conclude that genetic defects in the DNA mismatch repair system, responsible for the RER+ phenotype, do not play an important role in oesophageal cancers.

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