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Original Paper

Mutations in Mitochondrial Control Region DNA in Gastric Tumours of Japanese Patients

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The non-coding control region of mitochondrial DNA (mtDNA), containing the hypervariable regions HV1 and HV2 and the D-loop region, was screened for mutations in 45 gastric tumours (15 tumours each of adenoma, differentiated adenocarcinoma and undifferentiated carcinoma). We found mutations in two of the 45 tumours (4%); a 1 bp A deletion at nucleotide position 248 in a differentiated adenocarcinoma and a G to A transition at nucleotide position 16 129 in an adenoma. We also observed 10 polymorphisms, four of which were not previously recorded. Both mtDNA mutations were present in replication error negative (RER −) tumours. Short mono- or dinucleotide repeats in the control region, such as (C)7, (A)5 or (CA)5, were not altered regardless of nuclear genetic instability. In summary, mtDNA is mutated in a subset of benign and malignant gastric tumours, but, disruption of the mtDNA repair system appears not to be significantly involved in gastric tumours of Japanese patients.

Key words: mitochondrial DNA, mutation, gastric carcinoma
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INTRODUCTION

THE PATHOGENESIS of gastric carcinoma is complex and multifactorial, and it is clear that several genetic pathways exist in the development and progression of gastric carcinoma. Genetic alterations, such as *p53* gene mutations and loss of heterozygosity (LOH) at several chromosomal loci are known to be involved. However, incidences of such genetic abnormalities in gastric carcinomas rarely exceed 50%, and no gastric carcinoma-specific mutations have been identified [1, 2]. Genetic instability associated with the replication error (RER) phenotype, caused by disruption of the DNA mismatch repair system, is also present in a subset of gastric carcinoma and is probably involved in the progressive stage of the disease [3, 4].

Mitochondrial DNA (mtDNA) has no protective histones, a less effective repair system and is highly exposed to the oxygen free radicals generated by oxidative phosphorylation [5]. Thus, the mutation rate for mtDNA is around 10 times higher than that of nuclear DNA [5]. Mutations in the coding regions of mtDNA are associated with neurological diseases and diabetes [6, 7]. The mtDNA non-coding region, which contains hypervariable regions HV1 and HV2, origin of replication, the D-loop region and both origins of transcription, exhibits a high degree of sequence polymorphisms [8, 9]. While it is assumed that the sequence polymorphisms are not functionally relevant, the frequency and type of mtDNA mutations may reflect underlying genetic or environmental factors. In addition, mtDNA mutations may be sensitive biomarkers for environmentally induced genetic damage because of its high mutational rate. In this study, we screened for somatic mutations in the mtDNA control region of gastric adenoma and adenocarcinoma to determine if the mutational rate increases during gastric tumorigenesis.

MATERIALS AND METHODS

DNA was extracted from fresh or frozen samples of matched tumour and normal DNA of 45 Japanese patients (15 tumours each of adenoma, differentiated adenocarcinoma and undifferentiated carcinoma) by the standard phenol/chloroform extraction procedure [10]. All the samples contained more than 50% tumour cells histologically. The adenoma and differentiated adenocarcinoma DNA samples were

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Table 1. *Polymorphisms in the mitochondrial DNA control region*

Nucleotide position	Published sequence [8]	Polymorphism
101	G	A
150	C	T*
204–207	TGTG	TG
303–309	(C)7	(C)9*
311–315	(C)5	C)6*
489	C	T*
514–523	(CA)5	(CA)4*
16 189	T	C*
16 325	T	C
16 399	A	G

*Previously reported [9, 11–14].

selected from DNA panels used in previous allelic and microsatellite analyses [2, 4]. Eight of the differentiated adenocarcinomas were known to exhibit the RER+ phenotype at multiple microsatellite loci. RER was not detected at any loci in the remaining samples. The undifferentiated carcinoma DNA samples had not been analysed for the presence of the RER+ phenotype. Therefore, we examined microsatellites *D2S123*, *D4S404* and *D6S265* by the method described previously [4], and were unable to detect RER at these loci.

The non-coding mtDNA control region, containing the hypervariable regions (HV1 and HV2) and the D-loop region, was screened for mutations by polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis followed by direct sequencing. Three sets of primers were used for PCR-SSCP and direct sequencing: 5′-CACCATTAGCACCCAAAGCT-3′ and 5′-TGATTTCACGGAGGATGGTG-3′, 5′-CTCACGGGAGCTCTCCATGC-3′ and 5′-CTGTTAAAAGTGCATACCGCCA-3′, 5′-CTAACACCAGCCTAACCAGA-3′ and 5′-GGGGTGATGTGAGCCCGTCT-3′. The PCR products for the HV1, HV2 and D-loop regions are 443 bp, 402 bp and 270 bp in length, respectively. PCR was performed for 35 cycles in a thermal cycler (Perkin-Elmer Cetus Co., Norwalk, Connecticut, U.S.A.) consisting of denaturation at 95°C for 30 sec, annealing at 55°C for 30 sec, and extension at 72°C for 1 min. Products were labelled with [α-^{32}P] during PCR and subjected to SSCP described previously [4]. Bands exhibiting variable patterns of mobility were separated and subjected to PCR using the same primers as those used in the prior PCR. The PCR products were purified and sequenced using a terminator cycle sequencing kit (Taq DyeDeoxy; Applied Biosystems, Foster City, California, U.S.A.) and a DNA sequencer (type 373A).

RESULTS

Only two mutations were detected in the tumour mtDNA samples tested: an A at nucleotide position 248 was deleted in a differentiated adenocarcinoma sample (Figure 1), and a G to A transition occurred at nucleotide position 16 129 in an adenoma sample (Figure 2). Faint mutant bands with the presence of wild-type bands were observed in these tumour mtDNA samples in SSCP (Figures 1 and 2), and these samples histologically contained more than 90% tumour cells. These observations indicated the heteroplasmic state of these mutations. We also detected 10 sequence polymorphisms, four of which were not previously reported (Table 1) [9, 11–14].

DISCUSSION

Although it is unlikely that mutations in the mtDNA control region are immediately deleterious or specifically contribute to tumorigenesis, the frequency or type of mtDNA mutations may reflect underlying genetic and environmental influences [12]. It is also possible that sequence variants in the mtDNA control region could influence disease-associated mutations in the coding regions [15]. Because of the high

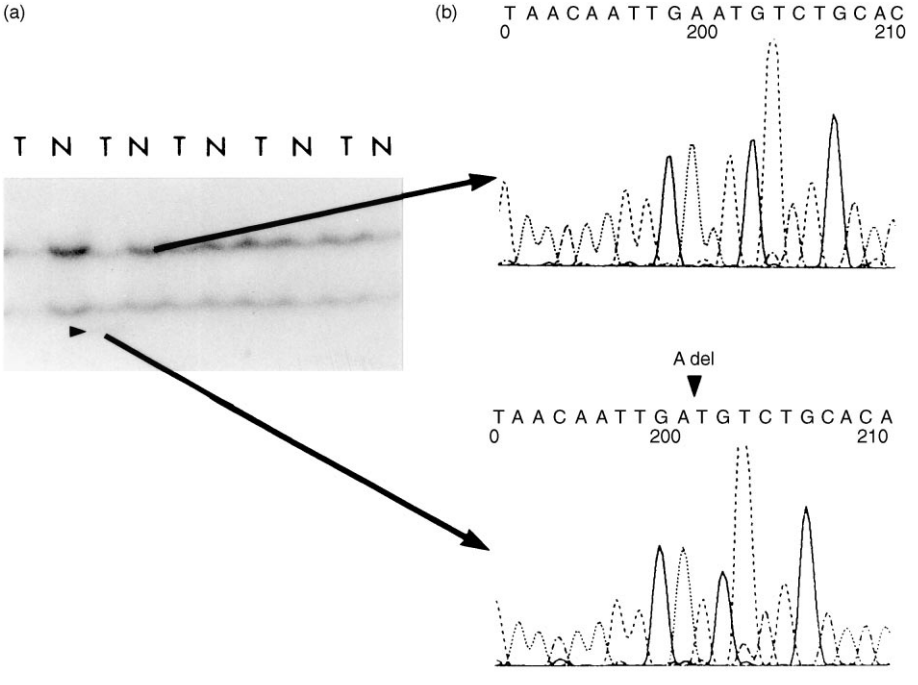

Figure 1. Representative PCR-SSCP analysis of the HV2 region (a) and sequencing histograms (b) in matched tumour and normal DNAs from gastric cancer patients. A differentiated adenocarcinoma carried a 1bp A deletion at nucleotide position 248. An arrowhead indicates a mobility shift band in tumour DNA. T, tumour DNA; N, normal DNA.

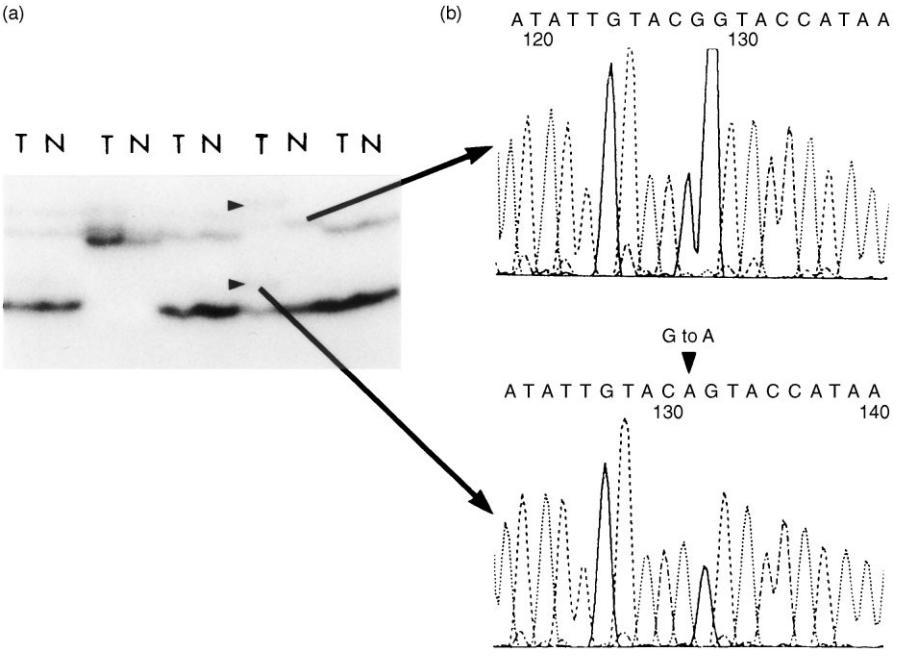

Figure 2. Representative PCR-SSCP analysis of the HV1 region (a) and sequencing histograms (b) in matched tumour and normal DNAs from gastric cancer patients. An adenoma carried a G to A transition at nucleotide position 16129. Arrowheads indicate mobility shift bands in tumour DNA. T, tumour DNA; N, normal DNA.

mutational rate of mtDNA, the presence of mutations may be a sensitive biomarker for environmentally induced genetic damage, which may develop into the early stages of clonal tumour growth. However, little is known about mtDNA mutation status in gastric tumours with only a few reports published [12, 13]. Burgart and collegues [12] analysed somatic mutations in the D-loop region of 77 gastric adenocarcinomas of American patients and found a 50-bp deletion in four samples (5.2%), all of which arose at the gastro-oesophageal junction. More recently, frequent mutations in HV1 (nucleotide position 16 024–16 365) and HV2 (nucleotide position 73–340) were reported to be present in 37% (3/8) of gastric and 23% (3/13) of colorectal adenocarcinomas of Spanish patients [13]. Four of the seven observed mutations (one gastric carcinoma carried two mutations) were located within runs of Cs or As [13]. Alonso and associates [13] suggested different mechanisms, such as clonal expansion, increased oxidative damage and nuclear mutator mutations, to explain the increased mtDNA mutation. Because most tumours are a clonal expansion of a single cell, it is possible that mtDNA mutations are just the results of clonal expansion of spontaneous somatic mutations that occurred at a very low frequency (not detectable) during previous replication of this precursor cell [16]. The hundreds to thousands of mitochondrial genomes per cell complicate interpretation since a mitochondrial mutation occurs in a single genome and later becomes apparent both by clonal expansion of its cell and by becoming homoplasmic or at least somewhat predominant through heteroplasmy within the cell. Even if some of the mtDNA mutations are just the results of clonal expansion, we have found differences in the frequencies or types of mtDNA mutations between previous reports [12, 13] and ours, although similar PCR-SSCP analyses were employed in these studies. In the present study, mutations of the mtDNA control region including HV1, HV2 and the D-loop were detected in two of 45 (4%) gastric tumours. The mutations were a 1bp A deletion in a differentiated adenocarcinoma and a G to A transition in an adenoma. Although our series contained eight carcinomas which developed in the cardiac portion (six differentiated and two undifferentiated carcinomas), the 50 bp deletions in the D-loop region were not detected. Short mono- or dinucleotide repeats in the control region, such as (C)7, (A)5 or (CA)5, were not altered. If the repeat sequence alterations are the results of mutator gene mutations as suggested by Alonso and associates [13], these mutations are less frequent in gastric carcinomas of Japanese patients. Thus, there may exist a significant ethnic difference in the pattern of mtDNA mutations that may reflect environmental factors. The mtDNA mutations detected in our study were present in RER – tumours. Heerdt and colleagues [11] sequenced the mtDNA D-loop region containing a (CA)5 repeat in colonic tumours and found no somatic mutations regardless of nuclear genetic instability. This and our present findings concur with the results in yeast; that the mtDNA mismatch repair is not carried out by the MSH2 gene product, but by a different protein encoded by the MSH1 locus [17, 18].

Our findings suggest that mtDNA is mutated in a subset of benign and malignant gastric tumours, and that the disruption of the mtDNA repair system is not significantly involved in gastric tumours of Japanese patients.

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