Occurrence of large-scale mitochondrial DNA deletions in human colorectal cancer

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Abstract

Introduction: The aim of this study was to determine the mutation patterns of colon cancers through screening of different regions of mitochondrial DNA (mtDNA) in colon cancer patients.
Material and methods: In order to investigate whether deletions exist in the mitochondrial DNA of colon cancer patients, we used a PCR assay to assess the presence of large-scale deletions. We screened four regions of the mitochondrial genome by PCR amplification and Southern blot analysis followed by DNA sequencing. Previously, deficiency in mitochondrial complex I has been reported; therefore we focused on the region of mtDNA that encodes the genes of this complex.
Results: In 11 out of 90 patients, we found an 8.7 kb deletion. Large-scale deletions of mtDNA are common events that have been found to occur in human ageing and in patients with mitochondrial myopathies. Based on our results the mtDNA 8.7 kb deletion occurs in 12.2% of the colorectal cancer (CRC) samples.
Conclusions: As reactive oxygen species (ROS) are continuously generated by the respiratory chain, they may cause significant oxidative damage to mtDNA (for example mtDNA deletions or mutations) if not efficiently eliminated. Defective respiratory enzymes containing protein subunits encoded by the deleted mtDNA may further enhance free radical production, resulting in more profound oxidative damage in CRC patients.

Key words: mitochondrial DNA, colon cancer, large-scale deletion, 8.7 kb deletion.

Introduction

Colorectal cancer is one of the most common human malignancies in both genders. It is the third most common cause of cancer-related deaths in the world [1].

It has been reported that mitochondrial DNA (mtDNA) is more susceptible to mutation than nuclear DNA (nDNA) [1, 2] and is frequently mutated in different types of cancers [3-14], including 10 to 70% of colorectal carcinomas [3, 5, 15-18]. These findings suggest a potential role of the mitochondrial genome in tumour carcinogenesis.

Mitochondria are cytoplasmic organelles that generate energy in the form of ATP through oxidative phosphorylation (OXPHOS) [19]. Human mtDNA is a double stranded circular molecule of 16,569 nucleotides and contains 37 genes coding for two rRNAs, 22 tRNAs and 13 polypeptides. The copy number of mtDNA is around 10^3–10^4 per cell and the vast majority of copies

are identical at birth. Furthermore, mtDNA is known for having a high acquired mutation rate which is 10 times higher than that of nuclear genomic DNA. It is generally accepted that the high mutation rate of mtDNA is caused by lack of protective histones, inefficient DNA repair systems and continuous exposure to mutagenic effects of oxygen radicals generated by OXPHOS [20]. Mutations in mtDNA have been reported to occur in human cancers [21-26].

Material and methods

Control subjects and patients

A total of 90 blood samples of CRC patients were collected from the Department of Oncology, Sayedoalshohadae hospital in Isfahan (Iran). The patients consisted of 46 women and 44 men ranging in age from 26 to 78 years (mean age 54.8 years). All of them had well differentiated adenocarcinomas.

Demographic, clinical and tumour-related characteristics of patients were recorded based on their hospital documents. These parameters included gender, age at diagnosis, place and date of birth and tumour-related factors such as location, stage, degree of differentiation and mucus production.

All of these patients were interviewed to trace their family history of cancer including occurrence of malignancy in the family, type of cancer and the age at diagnosis of the affected family member.

In the families of fifteen patients we saw occurrence of malignancy but only one case had colon cancer history in his family.

The control group consisted of 33 Iranian people (14 men and 19 women; mean age 37.5 years), who visited the blood donor clinic in Tehran. They answered an extensive questionnaire regarding their current health and medical history, and we chose healthy individuals with neither apparent genetic or metabolic disorders nor any type of cancer.

DNA extraction

Total DNA was isolated according to standard methods (DNAfast, Genfanavaran, Tehran, Iran).

Multiplex PCR

Multiplex PCR was carried out using five sets of primers: PD1/PD2, PD1/PD5, PD3/PD4, PD5/PD3 and PD6/PD3 (Figure 1). PD1 primer (5'-GAACATACAAAA CCCACCCC-3') located at 5421–5440 bp and PD2 primer (5'-GGCGGGAGAAGTAGATTGAA-3') located at 5740-5721 bp of the mtDNA were used to amplify a 319 bp fragment in a rarely deleted region as an internal control in each sample. PD1 primer and PD5 primer (5'-TTGGCGTGAAGGTAGCGGAT-3') located at 15000–14981 bp were used to amplify an 850 bp region created by the 8.7 kb deletion. PD3 primer (5'-CTACGGTCAATGCTCTGAAA-3') located at 8161–8180 bp and PD4 primer (5'-GGTTGACCTGTTA-GGGTGAG-3') located at 13640–13621 bp of the mtDNA were used to amplify the region created by the 5 kb deletion. PD1 primer and PD4 primer, PD3 primer and PD6 primer (5'-GTGGTCAAGTATTTATG-GTA-3') located at 16150–16131 bp were used to amplify regions created by 7.5 kb and 7.4 kb deletions.

Southern blot analysis

Genomic DNA was digested overnight with BamHI and was electrophoresed on 0.6% agarose gel. After electrophoresis, DNA was denatured, neutralized and transferred to positively charged nylon membranes. Meanwhile, mt15340F primer (5'-ATTCTTGCACGAAACGGGATC-3') located at 15340-15360 bp and mt91R primer (5'-GCTCCGGCT-CCAGCGTCTCG-3') located at 110-91 bp of the mtDNA were used to amplify 1339 bp fragment from the D-loop region. This fragment was used as an mtDNA probe for Southern blotting. Southern blot analysis was performed using DIG DNA Labeling and Detection Kit (Cat. #11093657910, Roche Penzberg, Germany).

Sequencing

Deletion breakpoints were analyzed by direct sequencing of mtDNA fragments amplified by the PCR reactions using an ABI 3700 capillary sequencer. Sequences then were compared with a comprehensive mitochondrial databank.

Statistical analysis

Qualitative variables were compared by Fisher's exact probability test. A P value of less than 0.05 was considered to indicate a statistically significant difference.

Results

In the last decade, mtDNA mutations have been reported to be associated with development and progression of colorectal cancer, which is probably the most studied cancer type in the mitochondrial field.

Figure 1. Diagram shows primers and their position on mtDNA

It has also been reported that mitochondria are the site of initiation of apoptosis; therefore, mutation of its genome may play a causative role in cancer.

Moreover, another report showed that 70% of colon cancers examined displayed mitochondrial DNA mutations [27].

The percentage of deleted 8.7 kb in our colon cancer patients was 12.2%, which means this deletion was observed in 11 patients out of 90. Recently an 8.9 kb deletion in gastric cancer was also reported [28]. We found a ~5 kb deletion in 2 of our patients (Figure 2). These deletions in CRC patients may result in multiple respiratory chain deficiencies [29].

In the case of 8.7 kb deletion, the repeat sequence flanking the deletion breakpoint was confirmed in some samples by sequencing. It was a 9-bp direct repeat (CTACTCCTA) in 5472/5481–14131/14140 and the deletion breakpoint was between nucleotide positions (np) 5472 and 14140 (Figures 3A, 3B). The deleted and control band are shown in Figure 4. The overall characteristics of 90 colon cancer patients with different deletions are summarized in Table I.

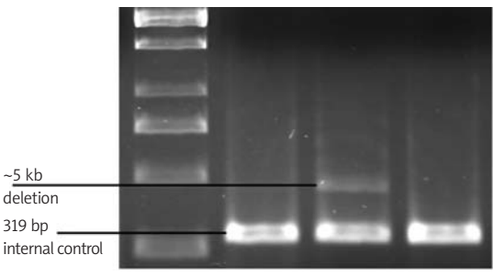

Figure 2. Detection of ~5 kb deletion in colon cancer by multiplex PCR

Table I. Characteristics of 90 colorectal cancer patients and frequency of the different deletions in them

	n	Frequency of ~8.7 kb deletion	Frequency of ~5 kb deletion
Patients	90	11	2
Controls	33	0	0
P value		0.035*	1
Age [years]:			
• **≥50**	64	9	2
• **<50**	26	2	0
Sex:			
• **male**	44	6	0
• **female**	46	5	2

**Significant*

Discussion

Deletion of ~8.7 kb causes a loss or truncation of the structural genes of ATPase 6/8, COIII, ND3, ND4L, ND4, ND5, ND6, Cytb and eight tRNA genes. Defective respiratory enzymes containing protein

A

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5361 tactccacct caatcacact actccccata tctaacaacg taaaaataaa atgacagttt gaacatacaa aacccacccc
5441 attcctcccc acactcatcg cccttaccac gctactccta cctatctccc cttttatact aataatctta tagaaattta
5521 ggttaaatac agaccaagag ccttcaaagc cctcagtaag ttgcaatact taatttctgt aacagctaag gactgcaaaa
     ..........................................................................................
14081 gcataattaa actttacttc ctctctttct tcttcccact catcctaacc ctactcctaa tcacataacc tattcccccg
14161 agcaatctca attacaatat atacaccaac aaacaatgTt caaccagtaa ctactactaa tcaacgccca taatcataca
14241 aagcccccgc accaatagga tcctcccgaa tCaaccctga cccctctcct tcataaatta ttcagcttcc tacactatta
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B

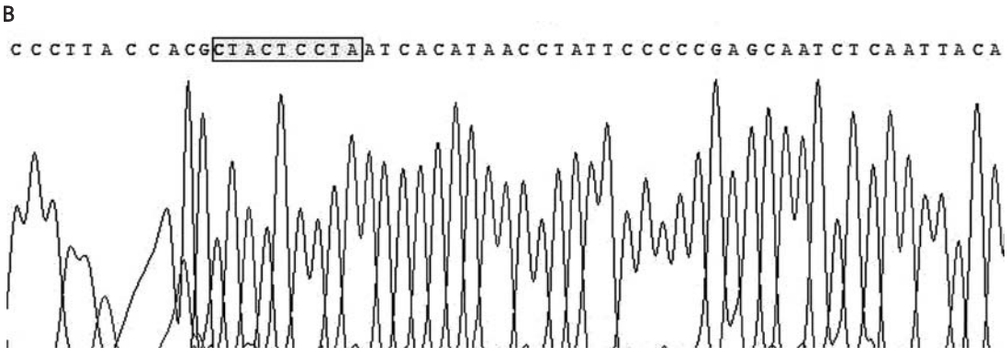

Figure 3. A – part of human mtDNA sequence. Denotation shows 9 bp directed repeat in 5472/5481–14131/14140. **B –** sequencing showing the 9 bp direct repeat flanking the ~8.7 kb deletion. Denotation show bases of repeat sequences

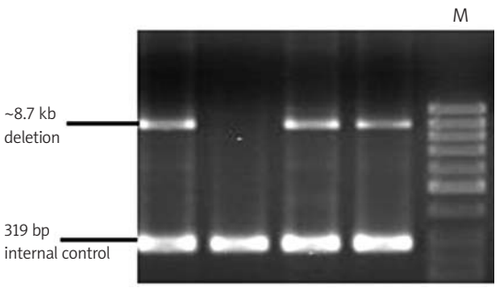

Figure 4. Detection of 8.7 kb deletion in colon cancer by multiplex PCR

subunits encoded by the deleted mtDNA may further enhance free radical production, resulting in more profound oxidative damage in patients. Beside the above fact, we know that the copy number of mtDNA is very high in cells and the consequences of deletion would be apparent when the deletion reached the threshold point.

In the presence of mtDNA deletions, which may be caused as mentioned by ROS or free radicals generated during aerobic metabolism, sensitive cells are deprived of ATP (due to the defective respiratory functions of mitochondria) and then they run into a state of energy crisis through a 'vicious cycle' as proposed by Wei [30]. This 'vicious cycle' may have catastrophic consequences and is accelerated by electron leakage from defective mitochondria; as such, it may play an important role in the pathophysiology of colorectal cancer patients.

Defective respiratory enzymes containing protein subunits encoded by the deleted mtDNA may further enhance free radical production, resulting in more profound oxidative damage in CRC patients. Since deletion in mtDNA is a sporadic event it could confirm the probable association between deletions in mtDNA and occurrence of colon cancer. As we demonstrate in Table I we did not find 8.7 kb deletions in our control subjects, which means that 8.7 kb deletions occur significantly more often in colon cancer patients than in the normal population.

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