

Mitochondrial haplogroup analysis in colorectal cancer: identification of a high-risk population

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Submitted: 14 March 2008

Accepted: 17 May 2008

Arch Med Sci 2008; 4, 4: 397–400
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Abstract

Introduction: Colorectal cancer is the third most common type of non-skin cancer in men (after prostate and lung cancer) and women (after breast and lung cancer). The mitochondrion conventionally is often thought to be an organelle specific to energy metabolism. It is in fact multifunctional and has been implicated in many diseases, including cancer. Alterations in the non-coding displacement loop of mitochondrial DNA are present in many types of cancer. In another word this loop has been shown to be a mutation “hot spot” in human cancers.

Material and methods: To assess the relationship between mitochondrial DNA haplogroups and colorectal cancer, we sequenced the mitochondrial DNA hypervariable segment I in a study population that comprised 95 cases (55 male, 40 female) and 100 unrelated healthy individuals as a control group. Haplotypes were assigned according to the West Eurasian mtDNA genealogy.

Results: We found that haplogroup K is more frequent in colorectal patients than healthy individuals. That means haplogroup K is significantly more abundant in colorectal cancer patients ($P=0.001$).

Conclusions: In this study we found a significant association of haplogroup K with colorectal cancer in Iranian patients so our finding suggests that mitochondrial genetic background plays a role in modifying an individual's risk for colorectal cancer.

Key words: mitochondrial DNA, haplogroup, colorectal cancer.

Introduction

Colorectal cancer (CRC) is a common lethal disease. CRC is the second leading cause of cancer death in both sexes, accounting for 10 to 11% of cancer deaths overall; it is the third most common in both men and women [1]. Approximately one in three people who develop CRC die of this disease.

A mitochondrial haplogroup is a cluster of phylogenetically related mitochondrial genotypes (haplotypes). These haplogroups are defined by ancient mutations [2]. These changes appeared and survived; therefore, they could not be deleterious mutations. Most of them probably did not have a phenotypic effect and were neutral. Some of them had a beneficial effect and were positively selected. However, this positive effect was related to a particular environment and nowadays, in other environmental conditions, may have different effects on the phenotype [1-3]. In others words human

haplogroups are defined by special polymorphisms in human mitochondrial DNA (mtDNA). These haplogroups trace the matrilineal inheritance of modern humans back to human origins in Africa and the subsequent spread across the globe.

Haplogroups could have important implications for understanding the relationship between mutability of the mitochondrial genome and disease [4, 5]. There is growing evidence that certain mtDNA clusters are associated with distinct disorders [6, 7].

Human mtDNA is composed of a 16.6 kb, double-stranded, closed and circular DNA molecule. It is independent of the nuclear genome. Human mtDNA contains genes encoding 13 polypeptides involved in oxidative phosphorylation (OXPHOS), 2 rRNAs, and a set of 22 tRNAs essential for protein synthesis in mitochondria [8]. All of the mitochondrial protein coding genes encode subunits of the OXPHOS enzymes that are responsible for the energy generating pathway. In addition, mtDNA contains a non-coding region called a displacement loop (D-loop), which is involved in the control of replication and transcription of mtDNA [9]. The D-loop is a region of 1124 base pairs (position 16024-576 on the mtDNA), which acts as a promoter for both heavy and light strands of mtDNA and contains essential transcription and replication elements [9].

This loop has been shown to be a mutation "hot spot" in human cancers, and it contains two hypervariable segment (HVS-I at positions 16024-16383 and HVS-II at positions 57-372) [10]. Because the D-loop is involved in the control of replication and transcription of mtDNA, mutations in this region might cause a decrease in the copy number or alteration in gene expression of the mitochondrial genome, which would further deregulate mitochondrial metabolism and OXPHOS.

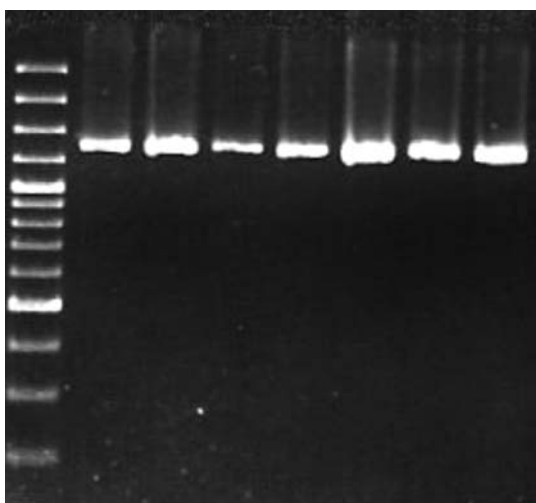


Figure 1. Demonstration of amplified 1339 bp D-loop region containing the HVS I region of a CRC patient. The HVS-I region of each CRC sample and control was amplified and directly sequenced and analyzed

Most of the mutations observed in both mtDNA coding and non-coding regions have occurred in pre-existing haplogroups and have defined the individual mtDNA types or haplotypes [11].

Material and methods

To investigate the involvement of mtDNA haplogroups in determining susceptibility to colon cancer, we sequenced the mtDNA HVS-I of 95 Iranian CRC patients. Also we sequenced 100 unrelated healthy individuals with neither apparent genetic or metabolic disorders nor any type of cancer as normal subjects (Table I).

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.

The local ethical committee approved the research proposal before beginning the project and all of the 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, and a consent form was taken from each patient to do this research.

The control group consisted of 100 unrelated Iranian people, 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. All of the patients and controls were informed of the aims of the study and gave their informed consent to the genetic analysis. The mean age was 49.3 for patients and 45.6 for normal controls. Peripheral blood samples were obtained and DNA was purified after lyses of white blood cells by use of a DNA extraction kit (DNAfast DNA Extraction Kit, Genefanavar, Tehran, Iran).

PCR amplification was carried out in a final volume of 25 μ l containing 200-300 ng total DNA, 70 μ M of each dNTP, 10 pmol of each primer, 2.5 mM MgCl₂, 1 U of *Taq* DNA polymerase (Cinnagen, Tehran, Iran) and 2.5 μ l of PCR buffer. The PCR profile was as follows: 94°C for 5 min, 30 cycles of 94°C for 50 s, 57°C for 50 s and 72°C for 50 s, followed by 72°C for 10 min. The sequenced products were analyzed on 1.5% agarose gel. The amplified sequences of some samples which demonstrate the 1339 bp fragment of the D loop region are shown in Figure 1.

Meanwhile, mt15340F primer (5'-ATTCTTGACGCA AACGGGATC -3') located at 15340-15360 bp and mt91R primer (5'-GCTCCGGCTCCAGCGTCTCG -3') located at 110-91 bp of the mtDNA were used to amplify a 1339 bp sequence encompassing HVS-I in the D-loop of the mtDNA to fetch the 359 bp sequence (16,024–16,383 nt) for HVS-I. The nucleotide

sequence of the amplicon was directly determined by automated sequencing on an ABI 3700 machine, using primer mt91R (Gene Fanavaran, Macrogene Seoul, Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL X with comparison to revised Cambridge Reference Sequence (rCRS) (<http://www.gen.emory.edu/mitomap/mitoseq.html>).

Haplotypes were assigned according to the West Eurasian mtDNA genealogy [12]. Assignment of the haplogroups is carried out using the following algorithm (for brevity all numbering is according to ref. [13] minus 16,000 in the control region of mtDNA): 069T 126C 223C assigned to haplogroup J; 126C 223C 294T assigned to T; 129A 223T 391A assigned to I; 223T 292T assigned to W; 189C 223T 278T assigned to X; 223C 224C 311C assigned to K; 362C assigned to D; 290T and 319A assigned to A; 223T assigned to R; 304C assigned to H1, 189C and 356T assigned to H3, 129A assigned to H4, 221T assigned to H5; 162G assigned to H8; 223C 249C and either 189C or 327T assigned to U1; 129C 223C assigned to U2; 223C 343G assigned to U3; 223C 356C assigned to U4; 223C 270T assigned to U5; 172C 219G 223C assigned to U6; 223C 318T assigned to U7; 223C 298C assigned to V; 223T 278T 390A assigned to L2; and 187T 189C 223T 278T 311C assigned to L1.

Statistical analysis

Fisher's exact probability test was used to examine the association between the two groups. A P-value of less than 0.05 determines the statistical significance of the relationship between CRC and the proportion of mtDNA with the mitochondrial haplogroups.

Results

Since mitochondria play pivotal roles in carcinogenesis and metabolism of cancer cells [12], we analyzed the correlation between cancers and mitochondrial haplogroups. We examined the relationship between colorectal cancer and each of 9 major mitochondrial haplogroups in Iranian CRC patients.

The mtDNA haplogroups of 95 colon cancer patients and 100 control subjects were determined by direct sequencing of mtDNA HVS I.

The studies of mtDNA polymorphism were based either on sequence variation in the control region, usually only the HVS-I segment, or RFLP analysis of the coding region [12-14]. mtDNA polymorphisms are associated with numerous chronic diseases, including diabetes, Alzheimer's disease, Parkinson's disease, bipolar disorders, and cancer.

Our result for the haplogroups for 95 colon cancer patients and 100 normal subjects shows that haplogroup K is more frequent in CRC patients than

Table I. Distribution of mtDNA haplogroups among colon cancer patients and normal controls

mtDNA haplogroups	Colon cancer patients [%]	Control subjects [%]	P-value
H	4/95 (4.2)	7/100 (7)	0.538
D	8/95 (8.42)	13/100 (13)	0.359
L	12/95 (12.63)	15/100 (15)	0.682
J	13/95 (13.68)	19/100 (19)	0.339
T	10/95 (10.52)	15/100 (15)	0.396
U	6/95 (6.31)	3/100 (3)	0.321
K	9/95 (9.47)	0/100 (0)	0.001*
A	2/95 (2.10)	1/100 (1)	0.613
R	20/95 (21.05)	18/100 (18)	0.717
*Other	16/95 (16.84)	9/100 (9)	0.133

Table II. Characteristics of 95 colorectal cancer patients and 100 normal controls

Parameters	No. of patients	No. of controls
Age		
• ≤50	28	68
• >50	67	32
Sex		
• Male	55	72
• Female	40	28
Tumour differentiation	Adenocarcinoma	–

*Statistically significant; Others – haplogroup I, W, X, and V

healthy individuals. The results are demonstrated in Table I. In this table the distribution of different mtDNA haplogroups in the studied CRC patients as well as in the population of normal controls is listed and the statistical parameter of the P-values are calculated and shown.

The characteristics of the tested CRC patients and the normal controls including age and sex are depicted in Table II.

Discussion

As demonstrated in Table I, haplogroup K is significantly more abundant in CRC patients (P=0.001). This finding is suggestive of a significant role for mtDNA haplotype in cancer development and risk. Our data showed that patients with colorectal cancer clustered in haplogroup K have a significantly higher frequency when compared with controls, implicating a possible association of haplogroup K with colorectal cancer. We concluded that mitochondrial polymorphisms in haplogroup K might play a genetic role in predisposing to colorectal cancer. Substitutions in the D-loop may be part of a haplotype with mutations elsewhere in the mtDNA. Also mtDNA HVS-I mutations may cause energy deficiency in stressful situations during a vulnerable developmental period [15]. The hypothesis is that on their own some polymorphisms are selectively neutral,

but in specific combinations they act in a synergistic, deleterious manner with established pathogenic mtDNA mutations to increase the risk of disease expression or to produce a more severe clinical outcome. The rich variability within HVS-I compared with the relatively constant constellation within the gene regions provides useful criteria for pathogenetic studies. This is the first study to trace mtDNA HVSI variants in CRC patients of the Persian population. We concluded from the tested data that haplogroup K is considerably more frequent in CRC patients ($P=0.001$) (Table I). Thus, mtDNA haplogroup K might constitute a risk factor for colon cancer.

Our results for haplogroup K are consistent with a recent study reporting that individuals bearing haplogroup K have increased risk for breast cancer [16]. But it is interesting that another research group recently reported that mtDNA haplogroups K and J have an apparent protective effect on Parkinson's disease [17, 18]. Conversely, haplogroup J has also been found to increase the risk for disease expression of Leber's hereditary optic neuropathy [19]. In our study we observed that the frequency of haplogroup U in the control sample population is half of the frequency of haplogroup J in the patient population; however it is not statistically significant (P -value=0.321).

Also an increased risk for haplogroups K and A was previously reported by Iranian researchers for multiple sclerosis patients [20]. We did not find any relationship between clinical characteristics and haplogroup K in our patients.

In conclusion, our data suggest an association of haplogroup K with CRC in Iranian patients. However, more studies of all types of cancers of both genders with stratification of the data set by sex are necessary and also further investigations on haplotype and other genes must be performed to shed new light on the molecular pathogenesis of CRC.

In this contribution we found a significant association of haplogroup K with CRC in Iranian patients so it can be concluded that mtDNA haplogroup K might constitute a risk factor for colon cancer.

Acknowledgments

The authors would like to acknowledge the assistance and advice of M. Dehghan and M. Rostami. We also would like to thank patients and their families, whose collaboration and understanding allowed us to do this work. This research was supported by the Department of Genetics of the Special Medical Center, Tehran, Iran.

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