Original Article

Identification of polymorphisms in mitochondrial cytochrome c oxidase genes as risk factors for gastric cancer

Yingnan Wang[1], Hongcai Wang[1], Shice Yin[2], Jingjing Zhang[3], Ruixing Zhang[1], Zhanjun Guo[3]

[1]Department of Gastroenterology and Hepatology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China; [2]Department of Intensive-care Unit, The Xingtai People's Hospital, Xingtai, China; [3]Department of Rheumatology, The Fourth Hospital of Hebei Medical University, Shijiazhuang, China

Contributions: (I) Conception and design: Y Wang, H Wang; (II) Administrative support: R Zhang, Z Guo; (III) Provision of study materials or patients: R Zhang, Z Guo; (IV) Collection and assembly of data: Y Wang, H Wang, S Yin, J Zhang; (V) Data analysis and interpretation: Y Wang, H Wang, S Yin, J Zhang; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

Correspondence to: Ruixing Zhang, MD. Department of Gastroenterology and Hepatology, The Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang 050011, China. Email: zrx@medmail.com.cn; Zhanjun Guo, MD. Department of Rheumatology, The Fourth Hospital of Hebei Medical University, 12 Jiankang Road, Shijiazhuang 050011, China. Email: zjguo5886@aliyun.com.

Background: Single nucleotide polymorphisms (SNPs) in the D-Loop region of mitochondrial DNA (mtDNA) have been implied in tumorigenesis of different types of tumors, but the associations involving polymorphisms in mtDNA coding regions still need to be clarified. This study aimed to identify SNPs of mitochondrial cytochrome c oxidase genes (MT-CO) in the occurrence of gastric cancer (GC).

Methods: The MT-CO genes were sequenced between 170 GC patients and 174 matched healthy controls. The χ^2 test was used to analyze differences in SNP frequencies between the two groups.

Results: The SNPs of MT-CO region were associated with the risk of GC. The genotype 9540T was significantly associated with an increased risk for GC (P=0.018), whereas 9548G was associated with a reduced risk (P=0.029).

Conclusions: The SNPs in MT-CO genes were found to be risk biomarkers for GC. It may provide a novel insight into the molecular mechanism in GC tumorigenesis and progression.

Keywords: Gastric cancer (GC); mitochondrial cytochrome c oxidase (MT-CO); mitochondrial DNA (mtDNA)

Submitted Oct 19, 2019. Accepted for publication May 11, 2020.
doi: 10.21037/tcr-19-2227
View this article at: http://dx.doi.org/10.21037/tcr-19-2227

Introduction

Although the incidence of gastric cancer (GC) has declined substantially in the past few decades, it remains the fifth most common cancer and the third most frequent cause of cancer deaths worldwide (1,2). GC is multifactorial, and it is very important to develop reliable biomarkers for predicting the risk of GC to maximize therapeutic effects and to minimize adverse effects of treatment. Many studies have evaluated the roles of nuclear DNA alterations in gastric tumorigenesis; however, relatively less attention has been paid to mitochondrial DNA (mtDNA) alterations (3).

The proximity of the mitochondrial genome to reactive oxygen species production sites, limited repair mechanisms, and a lack of protective histone proteins all result in a higher mutation rate in the mitochondrial genome than in the nuclear genome (4). In the present study, we have identified associations between mutations in the D-Loop and a wide variety of cancers, including GC, colorectal cancer, non-Hodgkin's lymphoma, non-small cell lung cancer and breast cancer, etc., but associations involving polymorphisms in mtDNA coding regions remain largely unknown (5-9). Mitochondrial cytochrome c oxidase (MT-CO) genes (including MT-CO1, MT-CO2 and MT-CO3) encode three subunits of respiratory complex IV, a key enzyme in aerobic metabolism. Mutations in MT-CO genes may

Table 1 Primer pairs used to amplify the mitochondrial cytochrome c oxidase genes (MT-CO) region

Gene	Forward primer; reverse primer
MT-CO1 (5530-6050)	5'-GCTACTCCTACCTATCTCCC -3'; 5'- TGTGGTCGTTACCTAGAAGG-3'
MT-CO1 (6040-6530)	5'- CTATTATTCGGCGCATGAGC -3'; 5'- TTGAGGTTGCGGTCTGTTAG -3'
MT-CO1 (6550-7130)	5'- CCTATCTCTCCCAGTCCTAG -3'; 5'- GGATTTTGGCGTAGGTTTGG -3'
MT-CO2 (7120-7600)	5'- GCCATCATAGGAGGCTTCAT-3'; 5'- AGACCTACTTGCGCTGCATG -3'
MT-CO2 (7640-8180)	5'- ACATGCAGCGCAAGTAGGTC-3'; 5'- AACTGTGGTTTGCTCCACAG -3'
MT-CO2 (8200-8770)	5'- CACTTTCACCGCTACACGAC -3'; 5'- TCCGAGGAGGTTAGTTGTGG -3'
MT-CO3 (8870-9320)	5'- CCACAACTAACCTAATCGGA-3'; 5'- AGCGTTATGGAGTGGAAGTG -3'
MT-CO3 (9320-9810)	5'- TCTCAGCCCTCCTAATGACC-3'; 5'- TGACGTGAAGTCCGTGGAAG -3'
MT-CO3 (9640-10090)	5'-GTCCCACTCCTAAACACATC -3'; 5'-GTAAGGCTAGGAGGGTGTTG -3'

play important roles in cancer formation by increasing the production of reactive oxygen species during mitochondrial oxidative phosphorylation (10). We have previously found that single nucleotide polymorphisms (SNPs) in MT-CO genes are important in evaluating the risk of hepatocellular carcinoma (11). However, no studies have confirmed that SNPs in MT-CO genes have a good predictive value on GC.

In this study, we sequenced a region of approximately 4,560 bp flanking the majority of MT-CO genes from the blood of patients with GC to identify SNPs associated with cancer and these results may facilitate the precise prediction of the risk of gastric tumorigenesis. We present the following article/case in accordance with the STREGA reporting checklist (available at http://dx.doi.org/10.21037/tcr-19-2227).

Methods

Sample preparation and DNA extraction

Blood samples were obtained from 170 patients with GC, who underwent tumor resection in the Department of General Surgery in 2007–2008 at the Fourth Hospital of Hebei Medical University. Data were collected from each GC patient including gender, age at diagnosis, tumor size, extent of differentiation, and stage. Blood samples of 174 healthy subjects receiving a physical examination were also collected. All procedures were supervised and approved by the Human Tissue Research Committee at the hospital. The number of ethical approval was MEC2008-2. Informed consent was obtained from all participants before enrollment and all the samples were anonymous.

Total mtDNA was isolated from blood samples and cells using the Wizard® Genomic DNA Purification Kit (Promega Corporation, Fitchburg, WI, USA) according to manufacturer's instructions and immediately stored at –20 ℃.

PCR amplification and sequence analysis

The primer pairs for MT-CO1 (bp 5530–6050), MT-CO1 (bp 6040–6530), MT-CO1 (bp 6550–7130), MT-CO2 (bp 7120–7600), MT-CO2 (bp 7640–8180), MT-CO2 (bp 8200–8770), MT-CO3 (bp 8870–9320), MT-CO3 (bp 9320–9810), and MT-CO3 (bp 9640–10090) are listed in *Table 1*. PCR was performed using the PCR Green Master Mix (Thermo, Billerica, MA, USA) according to the manufacturer's instructions and PCR products were purified prior to sequencing. Reaction parameters were as follows: 95 ℃ for 3 min, followed by 35 cycles of 95 ℃ for 30 s, 55 ℃ for 30 s, and 72 ℃ for 1 min, and a final extension at 72 ℃ for 5 min. Cycle sequencing was performed using the Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), and the products were read using the ABI PRISM® 3100 Genetic Analyzer (Life Technologies, Carlsbad, CA, USA).

Statistical analysis

All the experimental results were calculated using SPSS 24.0 statistical software (SPSS Inc., Chicago, IL, USA). The associations between the SNPs in the MT-CO genes and clinical parameters and the risk of GC were assessed using chi-squared tests. The magnitude of the association was estimated by the odds ratio (OR) and 95% confidence intervals (95% CI). All assays were repeated for at least three times. $P<0.05$ was considered statistically significant, and all reported P values are two-sided.

Table 2 The clinical characteristics of cases and controls

Group	Case (n=170)[1]	Control (n=174)	P value
Age (years)			0.393
≤60	85	95	
>60	85	79	
Gender			0.075
Male	120	107	
Female	50	67	

[1]Sample size.

Table 3 Nine single nucleotide polymorphisms (SNPs) between 28 cases and controls

Gene	Allele	Case (n=28)[1]	Control (n=28)	χ^2	P value
MT-CO1	6392T/C	26/2	24/4	0.187	0.666
	6455C/T	24/4	25/3	0.000	1.000
	6962G/A	25/3	27/1	0.269	0.604
	7196C/A	26/2	27/1	0.000	1.000
MT-CO2	7853G/A	26/2	27/1	0.000	1.000
MT-CO3	9540T/C	19/9	12/16	3.541	0.060
	9548G/A	25/3	28/0	1.409	0.235
	9824T/C	24/4	27/1	0.878	0.349
	9950T/C	26/2	28/0	0.519	0.471

[1]Sample size.

Results

A total of 170 patients with GC and 174 healthy controls were enrolled in the study. There were no statistical differences in the SNP frequency distribution with respect to age and gender. This meant that the two groups of patients were comparable (*Table 2*).

We analyzed mitochondrial MT-CO1 (nucleotides 5904–7445), MT-CO2 (nucleotides 7586–8269), and MT-CO3 (nucleotides 9207–9990) sequences in 28 patients with GC and healthy controls randomly. Nine SNPs with a minor allele frequency exceeding 5% in either patients or controls were used for the cancer risk analysis (*Table 3*). Two potential cancer risk-associated SNPs, 9540T/C (P=0.060) and 9548G/A (P=0.235), determined by χ^2 tests were reevaluated using all subjects. Associations of the SNPs with GC are summarized in *Table 4*. The 9540T genotype was significantly associated with a higher risk of GC (P=0.018, OR =1.671, 95% CI: 1.090–2.561), and 9548G was significantly associated with a reduced risk (P=0.029, OR =0.208, 95% CI: 0.044–0.977).

The SNPs related to GC were compared with the clinical characteristics of patients. Data demonstrated that the SNP sites of 9540T/C was associated with age-at-onset of the patients. The age-at-onset for patients with 9540C genotype was significantly earlier than that of patients carrying 9540T (P=0.021). Other clinicopathological variables, such as gender, tumor size, extent of differentiation, and stage showed no significant correlation with nucleotides 9540T/C (*Table 5*). Additionally, there was no significant difference between 9548 allele related to the incidence of GC and the clinical characteristics. The results are shown in *Table 6*.

Discussion

Mitochondrial DNA is predicted to be involved in carcinogenesis owing to the high mutation rate and limited repair mechanisms. We previously focused on the role of

Table 4 The single nucleotide polymorphisms (SNPs) at positions 9,540 and 9,548 between cases and controls

Allele	Case (n=170)[1]	Control (n=174)	χ^2	P value	OR	95% CI
9540T/C	91/79	71/103	5.588	0.018	1.671	1.090–2.561
9548G/A	161/9	172/2	4.772	0.029	0.208	0.044–0.977

[1]Sample size.

Table 5 Alterations in alleles 9540 in relation to clinical characteristics of gastric cancer patients

Characteristics	No. of 9540T/Total	Percentage	P value
Gender			0.351
Male	67/120	55.8%	
Female	24/50	48.0%	
Age (years)			0.021
≤60	38/85	44.7%	
>60	53/85	62.4%	
Tumor size (diameter)			0.560
≤6 cm	42/82	51.2%	
>6 cm	49/88	55.7%	
Extent of differentiation			0.451
Moderately differentiated	34/68	50.0%	
Poorly differentiated	57/102	55.9%	
Clinical stages			0.153
I + II	31/50	62.0%	
III + IV	60/120	50.0%	

mitochondrial D-Loop variation in tumor development. In this study, we examined the roles of MT-CO genes in mtDNA coding regions and identified two SNPs at positions 9540 and 9548 associated with GC risk by χ^2 analysis. This is the first study to report an association between MT-CO genes and GC. In addition, the present study showed the age-at-onset for patients with 9540C genotype was significantly earlier than that of patients carrying 9540T. SNPs in the MT-CO genes may prove effective for predict age at onset in GC patients, which needs to be further researched in future.

Many cancer-associated mtDNA polymorphisms inhibit the oxidative phosphorylation of respiratory chain (12,13). The MT-CO genes encode three subunits of respiratory complex IV, which is the terminal enzyme in the electron transport chain that catalyzes the final step of electron transfer from reduced cytochrome c to oxygen to generate H_2O (14). Homoplasmic polymorphisms in this region are thought to be too subtle to have detectable effects on oxidative phosphorylation, but the long-term accumulation of subtle differences in oxidative phosphorylation activity may result in oxidative stress. Thus, mtDNA polymorphisms can have important roles in tumor formation. There are reports of associations of polymorphisms in mtDNA coding regions with human cancer (15). We previously identified an association between a SNP at nucleotide position 9545 and hepatocellular carcinoma risk (11). However, 9540T/C, 9545A/G, and 9548 G/A in MT-CO3 are synonymous substitutions. This does not exclude the possibility that the nucleotide substitutions cause impairments in RNA processing due to improper precursor RNA folding (16).

There are still some shortcomings due to the limited experimental conditions. For example, the significance of mutations in these genes for the occurrence and

Table 6 Alterations in alleles 9,548 in relation to clinical characteristics of gastric cancer patients

Characteristics	No. of 9548G/Total	Percentage	P value
Gender			0.627
Male	113/120	94.2%	
Female	48/50	96.0%	
Age (years)			0.304
≤60	82/85	96.5%	
>60	79/85	92.9%	
Tumor size (diameter)			0.109
≤6 cm	80/82	97.6%	
>6 cm	81/88	92.0%	
Extent of differentiation			0.263
Moderately differentiated	66/68	97.1%	
Poorly differentiated	95/102	93.1%	
Clinical stages			0.791
I + II	47/50	94.0%	
III + IV	114/120	95.0%	

development of cancer still needs to be verified by further research. A statistical analysis with big data cannot be performed due to limited experimental subjects and we will conduct a longer follow-up study on the subjects to obtain more valuable guidance.

Taken together, our results combined with those of previous studies suggest that genetic polymorphisms in MT-CO genes may be useful for identifying patients at high risk for developing GC. More extensive biochemical and molecular studies will be essential to determine the pathological significance of these changes.

Acknowledgments

Funding: This work was supported by Science and Technology Plan Projects of Hebei Province [Grant No. 162777114D] and Natural Science Foundation of Hebei Province [Grant No. H2015206461].

Footnote

Reporting Checklist: The authors have completed the STREGA reporting checklist. Available at http://dx.doi.org/10.21037/tcr-19-2227

Data Sharing Statement: available at http://dx.doi.org/10.21037/tcr-19-2227

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-19-2227). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures were supervised and approved by the Human Tissue Research Committee at the hospital. The number of ethical approval was MEC2008-2. Informed consent was obtained from all participants before enrollment and all the samples were anonymous. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018;68:394-424.
2. Wang SM, Zheng RS, Zhang SW, et al. Epidemiological characteristics of gastric cancer in China, 2015. Zhonghua Liu Xing Bing Xue Za Zhi 2019;40:1517-21.
3. Arakawa N, Sugai T, Habano W, et al. Genome-wide analysis of DNA copy number alterations in early and advanced gastric cancers. Mol Carcinog 2017;56:527-37.
4. Nguyen NNY, Kim SS, Jo YH. Deregulated mitochondrial DNA in diseases. DNA Cell Biol 2020. doi: 10.1089/dna.2019.5220.
5. Wang H, Wang Y, Zhao Q, et al. Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for gastric cancer. Mitochondrial DNA A DNA Mapp Seq Anal 2016;27:1045-7.
6. Guo Z, Zhao S, Fan H, et al. Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for colon cancer. Mitochondrial DNA A DNA Mapp Seq Anal 2016;27:4244-5.
7. Diao L, Wei G, Su H, et al. Sequence polymorphisms in the D-loop region of mitochondrial DNA and outcome of non-Hodgkin lymphoma. Mitochondrial DNA 2015;26:88-91.
8. Hu WX, Ding CM, Li RJ et al. Single nucleotide polymorphisms in the mitochondrial displacement loop and age-at-onset of non-small cell lung cancer. Genet Mol Res 2015;14:2512-7.
9. Lee H, Geng C, Cheng M, et al. Single nucleotide polymorphisms in the mitochondrial displacement loop and age-at-onset of familial breast cancer. Mitochondrial Dna A Dna Mappseq Anal 2016;27:3082-5.
10. Rak M, Bénit P, Chrétien D, et al. Mitochondrial cytochrome c oxidase deficiency. Clin Sci 2016;130:393-407.
11. Wang H, Xu J, Li D, et al. Identification of sequence polymorphisms in the mitochondrial cytochrome c oxidase genes as risk factors for hepatocellular carcinoma. J Clin Lab Anal 2018;32:e22299.
12. Moro L. Mitochondrial Dysfunction in Aging and Cancer. J Clin Med 2019;8:1983-98.
13. Zong WX, Rabinowitz JD, White E. Mitochondria and Cancer. Mol Cell 2016;61:667-76.
14. Douiev L, Abu-Libdeh B, Saada A. Cytochrome c oxidase deficiency, oxidative stress, possible antioxidant therapy and link to nuclear DNA damage. Eur J Hum Genet 2018;26:579-81.
15. Errichiello E, Balsamo A, Cerni M, et al. Mitochondrial variants in MT-CO2 and D-loop instability are involved in MUTYH-associated polyposis. J Mol Med 2015;93:1271-81.
16. Vivian CJ, Brinker AE, Graw S, et al. Mitochondrial genomic backgrounds affect nuclear DNA methylation and gene expression. Cancer Res 2017;77:6202-14.

Cite this article as: Wang Y, Wang H, Yin S, Zhang J, Zhang R, Guo Z. Identification of polymorphisms in mitochondrial cytochrome c oxidase genes as risk factors for gastric cancer. Transl Cancer Res 2020;9(6):3854-3859. doi: 10.21037/tcr-19-2227

