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ORIGINAL ARTICLE

Genetic variations of mitochondrial genome modify risk and prognosis of hepatocellular carcinoma patients

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Summary

Background: Previous studies have indicated that mitochondrial genetic variations were associated with the risk of many cancers. However, there are few reports on the association between single nucleotide polymorphisms (SNPs) or haplogroups of mitochondrial DNA (mtDNA) and the risk or prognosis of hepatocellular carcinoma (HCC).

Methods: In order to investigate the predictive and prognostic role of mtDNA SNPs and haplogroups in HCC, the mitochondrial genome of 188 HCC patients and 344 healthy controls were sequenced by next generation sequencing technology. Then, logistic regression analysis was used to determine the effect of mtDNA SNP or haplogroup on risk and prognosis of HCC patients.

Results: The haplogroup M7 had an odds ratio (OR) of 0.47 (95% CI = 0.24–0.91; $P = 0.026$) to develop HCC. The frequency of 152 T/C, 199 T/C, 4048G/A, 9824 T/C, 15784 T/C, 16185 C/T and 16399A/G was significantly different between HCC patients and the controls. In addition, multivariate analysis with COX hazards model showed that the patients with haplogroup M8 had lower

Abbreviations: HCC, hepatocellular carcinoma; SNPs, single nucleotide polymorphisms; HR, hazard ratio; CI, confidence interval.

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survival rate than the patients with haplogroup D4 (HR = 2.62, 95% CI = 1.03—6.68; $P = 0.044$). Three SNPs 15784 T/C, 16185 C/T and 16399A/G were also identified to have a statistically significant association with postoperative survival in HCC.
Conclusions: To date, these results provide the first evidence that mtDNA SNPs and haplogroups may be potential risk factors for susceptibility and survival of HCC patients.

Introduction

Hepatocellular carcinoma (HCC) is one of the most common cancers all over the world, and the mortality of HCC ranks the second in men and the sixth in women. Half of new cases occurs in China every year [1—3]. The prognosis of patients with HCC remains poor. Once HCC invades other organs and tissues or spreads distantly, the clinical outcome may be even worse [4]. Therefore, early detection and accurate assessment of HCC prognosis is of great importance. At present, the diagnosis of HCC mainly depends on imaging techniques, which are less efficient for the detection of early HCC [4]. Detection of plasma biomarker is an ideal method for screening patients at an early stage and predicting their prognosis. For example, plasma alpha fetoprotein (AFP) has been used most widely for HCC prognosis. However, it becomes less sensitive in detecting HCC at early stage and is limited by the false positivity in patients with active hepatitis. Thus, new tumor markers are urgently needed to detect the tumorigenesis and predict progression of HCC independently or act as a complement to AFP.

Mitochondria are ubiquitous organelles in eukaryotic cells with the primary function to generate energy in the form of ATP through oxidative phosphorylation [5]. Mitochondrion has its own genome (mtDNA), which replicates and inherits independently of the nuclear genome in a maternal-inherited pattern. mtDNA with multiple copies in one cell has a large number of mutations that have segregated during evolution. Interestingly, high level of mtDNA mutation has been detected in many kinds of cancers [6]. A human mtDNA haplogroup is defined by a unique set of mtDNA single nucleotide polymorphisms (SNPs), reflecting mutations accumulated by a discrete maternal lineage [7]. The haplogroups are associated with region-specific mtDNA sequence variations as a result of genetic drift and/or adaptive selection for an environment-favored mitochondrial function.

At present, it has been proved that mtDNA haplogroups are associated with risk or prognosis of a variety of cancers, such as colorectal cancer, breast cancer, gastric cancer, esophageal cancer and cervical cancer [8—12]. SNPs in mtDNA have also been linked to several types of cancers, such as gastric cancer [13], renal cell carcinoma [14], endometrial cancer [15], ovarian cancer [16] and breast cancer [17,18]. However, there are few studies about the relationship between mtDNA SNPs or haplogroups and the risk or outcomes of HCC.

In this study, we endeavored to detect all mtDNA genetic variations in a cohort of HCC patients, and matched healthy controls by whole mtDNA resequencing. Then, we assessed the association of mtDNA SNPs and haplogroups with risk and clinical outcome of HCC patients.

Materials and methods

Study population

Patients ($n = 188$) with primary HCC, which was diagnosed between January 2009 and January 2012, were recruited from the Eastern Hepatobiliary Surgery Hospital, Secondary Military Medical University (SMMU) in Shanghai, China. There was no recruitment restriction on age, gender and tumor stage and no previous history of other cancers for all patients. All patients received surgery within 2 months after diagnosis and no patient received other anticancer treatment before surgery. The study was approved by the Ethical Committees of SMMC and written consent was obtained from each patient. Sex-matched control samples ($n = 344$) were collected from public source [19,20]. All the subjects were Han nationality.

Demographic data were collected through in-person interview at the initial visit, follow-up in the clinics, medical chart review, or consultation with the treating physicians. The follow-up information was updated at 6-month intervals through onsite interview, direct call, or medical chart review. The latest follow-up in this study was carried out in January 2013. For each participant, 5 ml of venous blood was collected and used for genomic DNA extraction by using the E.Z.N.A. Blood DNA Midi Kit (Omega Bio-Tek, Norcross, GA, USA) in the laboratory.

Detection of mitochondrial genetic variations and haplogroup determination

Whole mitochondrial genomes were sequenced by Illumina Hiseq 2000 according to protocols suggested by the manufacturer. For each sample, clean read was aligned to reference genome (hg19 + rCRS) using BWA with default parameters. BAM file was sorted and indexed by Picard, with the duplicated reads to be removed by GATK. Reads that could properly align to rCRS with mapping quality above Q20 and base quality no less than Q30 were selected for further analysis. The consensus sequence was called from the major allele of each position by Samtools. Furthermore, the mtDNA haplogroup classification was determined according to the phylogenetic tree Build 16 of global human mtDNA (http://www.phylotree.org/) using the soft Mitotool [21].

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