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Relationship between mitochondrial DNA mutations and clinical characteristics in human lung cancer

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Abstract

Mitochondrial DNA (mtDNA) is known for its high frequencies of polymorphisms and mutations, some of which are related to various diseases, including cancers. However, roles of mutations and polymorphisms in some diseases are among heated debate, especially for cancer. To investigate the possible role of mtDNA mutations in lung cancer, we sequenced complete mtDNA of lung cancer tissues, corresponding normal (i.e., non-cancerous) lung tissues, and peripheral blood samples from 55 lung cancer patients and examined the relationship between mtDNA mutations or polymorphisms and clinical parameters. We identified 56 mutations in 33 (60%) of the 55 patients, including 48 point mutations, four single-nucleotide insertions, and four single-nucleotide deletions. Nineteen of these mutations resulted in amino acid substitution. These missense mtDNA mutations were distributed in 9 of 13 mitochondrial DNA coding genes. Three hundred eighty eight polymorphisms were identified among the 55 patients. Seventy-three polymorphisms resulted in amino acid substitution. There was no association of incidence of specific mtDNA mutation or polymorphism with patients' gender, age at diagnosis, smoking history, tumor type or tumor stage ($P > 0.05$). This study revealed a variety of mtDNA mutations and mtDNA polymorphisms in human lung cancer, some of which might be involved in human lung carcinogenesis.

Keywords: Mitochondrial DNA; Mutation; Lung cancer

1. Introduction

Human mitochondrial DNA (mtDNA) is a 16,569 base-pair (bp) circular double-stranded DNA molecule that encodes 13 polypeptides involved in oxidative phosphorylation, 22 transfer RNAs (tRNAs), and two ribosomal RNAs (rRNAs). Most cells contain 10^3–10^4 copies of mtDNA and the mutation rate of mtDNA is much higher than that of nuclear DNA (Wallace, 1994). The high copy number and the high mutation rate are two important features that distinguish mtDNA from nuclear DNA.

Mitochondrial dysfunction is increasingly recognized as an important cause of human pathology. There are now more than 50 disease-causing mtDNA mutations and hundreds of mtDNA rearrangement known (Simon and Johns, 1999; Wallace, 1999; Larsson and Luft, 1999). Over the past decade, many somatic mtDNA mutations have been identified in various tumor tissues and cell lines, including

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bladder cancers (Fliss et al., 2000), head and neck cancers (Fliss et al., 2000), lung cancers (Fliss et al., 2000), pancreatic cancers and pancreatic cancer cell line(Jones et al., 2001), hepatocellular carcinomas and hepatocellular carcinoma cell lines (Tamori et al., 2004; Nishikawa et al., 2001), colorectal cancers (Polyak et al., 1998; Habano et al., 1998), thyroid cancers (Abu-Amero et al., 2005; Yeh et al., 2000), breast cancers (Zhu et al., 2005; Rosson and Keshgegian, 2004; Richard et al., 2000), prostate cancer (Jessie et al., 2001; Petros et al., 2005), and gastric carcinomas (Habano et al., 2000). These findings have suggested two possibilities, that mtDNA mutations may be involved in the initiation of carcinogenesis or that such mutations could be used as biomarkers of carcinogenesis. It has been showed that mtDNA mutations may involved in carcinogenesis by changing cellular energy capacities, by increasing ROS, and/or by modulating apoptosis (Wallace, 1999; Penta et al., 2001; Brandon et al., 2006; Shidara et al.). Several groups have reported that in tumors cells, mtDNA mutations are easier to detect than nuclear DNA mutations; they concluded that mtDNA mutations may be attractive diagnostic markers for some cancers (Fliss et al., 2000; Jones et al., 2001; Isaacs et al., 2004). In our preliminary study (Jin et al., 2002), we found that mtDNA mutations were distributed in the whole mitochondrial genome of lung cancers. To further understand the possible relationship between mtDNA mutation and lung carcinogenesis, we analyzed the mtDNA sequence of lung cancers, corresponding normal (i.e., non-cancerous) lung tissues, and the peripheral blood of each patient. We also reviewed the clinic records of all patients collected in this study, and investigated the relationship of mtDNA mutations and patient's gender, age at diagnosis, or tumor type or grade.

2. Materials and methods

2.1. Patient selection

Fifty-five lung cancer patients diagnosed at the Department of Thorax, Cancer Hospital, Chinese Academy of Medical Sciences during the years of 1998–2000 were randomly selected for this study. All patients were operated on before they received any other treatment and their medical records were reviewed. The surgical specimens of lung cancers, including squamous cell carcinoma ($n = 29$), adenocarcinoma ($n = 15$), adenosquamous carcinoma ($n = 3$), small cell carcinoma ($n = 3$) and other types of tumors ($n = 5$), as well as the corresponding normal (i.e., non-cancerous) lung tissues and peripheral blood samples were collected and kept at −80 °C in our tissue bank. All cancer specimens and non-cancerous lung tissues were evaluated histologically by the pathologists of Cancer Hospital, Chinese Academy of Medical Sciences. This study was approved by the Institutional Review Board of the Cancer Institute (Hospital), Peking Union Medical College and Chinese Academy of Medical Sciences. All patients included in this study provided written informed consent.

2.2. Cell culture

The ρ^0 cells are human devoid of the mitochondria DNA and were a gift from Dr. Attardi (Division of Biology, California Institute of Technology, Pasadena, CA) (King and Attardi, 1989). Cells were cultured in Dulbecco's Modified Eagles's Media (DMEM) with 10% fetal bovine serum. Total DNA was extracted as previously described (Zhang et al., 2001). The total DNA from the ρ^0 cell line was used to ensure that only mtDNA was amplified from the patient tissue samples.

2.3. DNA extraction from patient samples

Harvested tumor tissues and distal normal lung tissues were frozen and sectioned with the use of a cryostat. Peripheral lymphocytes were isolated from blood as described previously (Zhang et al., 2001). Total DNA (i.e., mtDNA and nuclear DNA) was extracted from each tissue or blood sample as described previously (Zhang et al., 2001).

2.4. Polymerase chain reaction (PCR) amplification of mtDNA

We synthesized the 58 sets of unique primers whose sequences was previously described (Levin et al., 1999) and used them to amplify and sequence the entire 16,569-bp mtDNA genome in each sample of tissue and lymphocytes. Because the nuclear genome contains many sequences that share some homology with mtDNA, we first amplified the mitochondrial genome from each sample as a series of 14 overlapping 1.5–3-kilobase (kb) polymerase chain reaction (PCR) products to avoid amplification of these nuclear mitochondrial-like sequences (Parfait et al., 1998; Fliss et al., 2000). Each of those reaction mixtures contained 15 ng total DNA, 1 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), 2 μL 10× buffer II (Applied Biosystems, Foster City, CA, USA), 0.1 mM of each deoxyribonucleoside triphosphates (dNTPs), and 0.1 μM each of the appropriate forward and reverse primers in final volume of 20 μL. Thermal cycling was conducted with the use of a Perkin-Elmer Model 9700 thermocycler (Applied Biosystems, Foster City, CA, USA) and consisted of 10 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at 56 °C, and 2 min at 72 °C, followed by a final extension step of 10 min at 72 °C. We then performed a second round of PCR using the same reaction mixture as above with 0.5 μL of the first-round PCR products as templates and unique mtDNA primers. That PCR program consisted of an initial incubation of 10 min at 95 °C, followed by 25 cycles of 30 s at 95 °C, 30 s at 56 °C, and 1 min at 72 °C, followed by a final extension step of 10 min at 72 °C. The second-round PCR