MUTATIONAL ANALYSIS OF MITOCHONDRIAL tRNA GENES IN PATIENTS WITH LUNG CANCER

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ABSTRACT

Mutations in mitochondrial tRNA (mt-tRNA) genes have been found to be associated with various diseases including lung cancer. To understand the possible relationship between mtRNA mutations and lung cancer, we sequenced the 22 mt-tRNA genes from 200 lung cancer blood samples, as well as 100 healthy subjects. As a result, five mutations were identified including the tRNA\textsubscript{Ala} T5655C, tRNA\textsubscript{Arg} T10454C, tRNALeu(CUN) A12330G, tRNA\textsubscript{Ser(UCN)} T7505C and tRNAThr G15927A. These mutations were absent in the healthy subjects. These mutations and polymorphisms were localized at the highly conserved nucleotides of the corresponding mitochondrial tRNAs, which are critical for the tRNA steady state level and may result in failure in the tRNA metabolism. Moreover, through the application of the pathogenicity scoring system, we found that only the T10454C mutation should be classified as a “neutral polymorphism,” while the other mutations were regarded as “definitely pathogenic.” Taken together, our data indicate that tRNA genes are the hot-spots for pathogenic mutations associated with lung cancer. Our findings may provide valuable information for pathophysiology, management and genetic counseling of lung cancer.

Keywords: Lung cancer; Mitochondrial tRNA (mt-tRNA); Mutations; Pathogenic.

INTRODUCTION

Over the last century, lung cancer from the rarest of diseases became the biggest cancer killer of men worldwide and in some parts of the world also of women. It is the most common type of cancer diagnosed in the world and is the number one cancer killer in the US [1]. Survival rates in patients with lung cancer are much lower than patients with other common cancers, such as breast, colon or prostate cancer. It is estimated that by 2035, the number of lung cancer deaths will increase globally by 86.0% when compared to 2012 [2]. However, to date, the molecular mechanism underlying this disease remains poorly understood.

Mitochondria are cellular organelles with distinct features that belie their origins and unique functions. Originally derived from ancient aerobic bacteria, mitochondria are critical for meeting cellular energy demands by driving the synthesis of ATP [3]. Mitochondria also influence cellular signaling and survival pathways, including apoptosis [4]. Mitochondrial dysfunction has been implicated in a plethora of human diseases, most notably in cancer [5]. In addition, due to the lack of protective histones, introns and efficient DNA repair systems, mitochondrial DNA (mtDNA) acquires 10-fold more mutations than nuclear genomic DNA [6]. Among these mutations, mitochondrial tRNA (mt-tRNA) genes are hot-spots for pathological mutations and over 200 mt-tRNA mutations have been linked to various disease states [7,8]. Nevertheless, little is known regarding the mt-tRNA mutations in lung cancer.

In this study, we performed a systematic and extensive mutational screening for 22 mt-tRNA genes with lung cancer. We also used the pathogenicity scoring system for these mutations.
MATERIALS AND METHODS

Blood Samples. Since January 2015, a total of 200 lung cancer patients (45.0% females and 55.0% males, aged 45 to 55 years) were recruited from the First Affiliated Hospital, Soochow University, Suzhou, People’s Republic of China (PRC). Moreover, 100 unrelated healthy controls, age- and gender-matched, were collected in the same area. Blood and experimental procedures were approved by the Ethics Committee of Soochow University, Suzhou, PRC. A signed informed consent was obtained from all participants.

DNA Extraction, Polymerase Chain Reaction (PCR) Amplification and Sequence Analysis. The genomic DNA was extracted using standard phenol/chloroform methodology, and stored at –20 °C for future use. The 22 mt-tRNA genes were amplified by PCR, the primers information are listed in Table 1. The PCR mixture included 200 mm dNTP, 10X buffer, Taq DNA polymerase and 15 mM Mg2+ (Takara Biotechnology Co., Ltd., Dalian, China). Each amplified DNA sample was purified and analyzed using the ABI PRISM® 3700 automated DNA sequencer and the BigDye Terminator Cycle sequencing reaction kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA). The sequence data were compared with the reversed consensus Cambridge sequence to screen mutations (GenBank Accession No. NC_012920) [9].

Phylogenetic Conservation Analysis. A total of 16 vertebrates’ mtDNA sequences were used in the interspecific analysis. These included: Bos Taurus, Cebus albifrons, Gorilla gorilla, Homo sapiens, Hylobates lar, Lemur catta, Macaca mulatta, Macaca sylvanus, Musculus, Nycticebus coucang, Pan paniscus, Pan troglodytes, Pongo pygmaeus, Pongo abelii, Papio hamadryas and Tarsius bancanus. The conservation index (CI) was then calculated by comparing the human nucleotide variants with another 15 vertebrates. The CI was then defined as the percentage of species from the list of 15 different vertebrates that have the wild-type nucleotide at that position.

Pathogenicity Scoring System for These Mitochondrial tRNA Mutations. McFarland et al. [10] provided a program for assigning pathogenicity to mt-tRNA mutations. Their weighting scoring system was revised in 2011 [11]. According to this standard, we classified a mutation with a score of ≤6 as a “neutral polymorphism,” while a mutation with a score of 7-10 was a “possible pathogenic,” and a mutation with a score of >13 points was classed as “definitely pathogenic.”

RESULTS

Mutational Analysis of the Mitochondrial tRNA Genes. Mutational screening of the 22 mt-tRNA genes led us to identify five potential pathogenic mutations: tRNAGlu T5655C, tRNAVal T10454C, tRNAAsnUCN A12330G, tRNATyrUCN T7505C and tRNAGlu G15927A. Of these, the T5655C mutation was detected in one out of 200 patients (0.5%), the T10454C mutation was detected in two patients (1.0%), the A12330G mutation was detected in three patients (1.5%), the T7505C mutation was presented in one patient (0.5%) and the G15927A mutation was presented in four patients (2.0%). However, none of these mutations were found in the healthy controls. The characterization of each mt-tRNA mutation is listed in Table 1 and Figure 1.

Phylogenetic Conservation Analysis. To see whether these mutations contributed to the genetic susceptibility of lung cancer, we performed the phylogenetic conservation analysis for each mt-tRNA mutations. We found that all these mutations exhibited high levels of CI (100.0%), indicating that these mutations may play important roles in lung cancer.

The Pathogenicity Scoring System for These Mitochondrial tRNA Mutations. According to the revised pathogenicity scoring system, we found that the total score of the T5655C, T10454C, A12330G, T7505C and G15927A mutations were 11, 6, 14, 11 and 15 points, respectively, it seemed that except for the T10454C mutation, other mutations should be classified as “definitely pathogenic” (Table 2).

| tRNA Species | Sequence Alteration | Location | Conservation Index (%) | Frequency (%) |
|--------------|---------------------|----------|------------------------|--------------|
| tRNAAsn     | T5655C              | Acceptor arm | 100.0                  | 0.5          |
| tRNAVal     | T10454C             | T loop    | 100.0                  | 1.0          |
| tRNATyrUCN  | A12330G             | Acceptor arm | 100.0                  | 1.5          |
| tRNATyrUCN  | T7505C              | DHU stem  | 100.0                  | 0.5          |
| tRNAGlu     | G15927A             | Anticodon stem | 100.0                  | 2.0          |
Table 2. The pathogenicity scoring system for the mt-tRNA mutations for lung cancer.

| Scoring Criteria                                      | T5655C | T10454C | A12330G | T7505C | G15927A | Classification |
|-------------------------------------------------------|--------|---------|---------|--------|---------|----------------|
| More than one independent report                      | yes    | yes     | yes     | yes    | yes     | 2              |
| Evolutionary conservation of the bp                   | no     | no      | no      | no     | no      | ≥6 points: neutral polymorphisms |
| Variant heteroplasmy                                  | no     | no      | no      | no     | no      | 7-10 points: possibly pathogenic |
| Segregation of the mutation with disease              | yes    | yes     | yes     | yes    | yes     | 2              |
| Histochemical evidence of mt disease                  | no     | no      | yes     | no     | no      | 11 points: definitely pathogenic |
| Biochemical defect in complexes I, III or IV          | no     | no      | yes     | no     | yes     | 2              |
| Evidence of mutation segregation with biochemical defect from single-fiber studies | no     | no      | yes     | no     | yes     | 2              |
| Mutant tRNA steady-state level of evidence of pathogenicity in trans-mt cybrid studies | strong evidence | no | no | strong evidence | 5 strong evidence | 5 |
| Maximum score                                         | definitely pathogenic | neutral polymorphism | definitely pathogenic | definitely pathogenic | definitely pathogenic | 15 |

bp: base pair; mt: mitochondrial.

* Score out of 20.

Figure 1. Cloverleaf structure of mt-tRNA<sup>Ala</sup>, mt-tRNA<sup>Arg</sup>, mt-tRNA<sup>Leu(CUN)</sup>, mt-tRNA<sup>Ser(UCN)</sup> and mt-tRNA<sup>Thr</sup>. The arrows indicate the position of the T5655C, T10454C, A12330G, T7505C and G15927A mutations.
DISCUSSION

In this study, we screened mt-tRNA mutations in patients with lung cancer. To the best of our knowledge, this is the first report dealing with the association between mt-tRNA mutations and lung cancer. Lung cancer is a common type of cancer diagnosed all over the world. Screening options for this disease are very limited and only 15.0% of cases are diagnosed at an early stage [12]. Thus, there is an urgent need for developing a biomarker for early detection for lung cancer.

Mitochondrial tRNA genes are hot-spots for pathological mutations and over 200 mt-tRNA mutations have been linked to various disease states. Often these mutations prevent tRNA aminoacylation, disrupting this primary function affects protein synthesis and expression, folding and function of oxidative phosphorylation enzymes [13]. However, we noticed that a certain amount of tRNA mutations have been wrongly classified as “pathogenic,” as in a recent article concerning the association between mt-tRNA mutations and thyroid carcinoma [14].

In this study, we investigated the possible relationship between mt-tRNA mutations and lung cancer by employing PCR-Sanger sequencing. As a result, five mt-tRNA mutations were identified, including tRNA\textsuperscript{Ala} T5655C, tRNA\textsuperscript{Ala} T10454C, tRNA\textsuperscript{Ser(UCN)} A12330G, tRNA\textsuperscript{Ser(UCN)} T7505C and tRNA\textsuperscript{Thr} G15927A. Of these, the T5655C mutation was located at the acceptor arm of tRNA\textsuperscript{Ala}, disrupted the highly conserved base-pairing (1A-72U). An in vitro processing analysis showed that the T5655C mutation reduced the efficiency of tRNA\textsuperscript{Ala} precursor S' end cleavage catalyzed by RNase P [15]. Moreover, a significant decreased of tRNA\textsuperscript{Ala} steady-state level was also observed in hybrid cells containing this mutation. While the homoplasmic T10454C mutation was localized at the T loop of tRNA\textsuperscript{Ala} (conventional position 55), this mutation was implicated to be associated with longevity and non syndromic hearing loss [16,17]. However, a previous study showed that the T10454C mutation may not modulate the phenotypic manifestation of deafness-associated mitochondrial 12S rRNA A1555G mutation [18], thus, we proposed that the T10454C mutation was a neutral polymorphism (Table 2). In addition, the A12330G mutation occurred at the acceptor arm of tRNA\textsuperscript{Ser(UCN)} gene, disrupting the highly conserved base-pairing (6T-67A), and may result in failure in tRNA metabolism. A previous study showed that this mutation, combined with the well known ND5 T12338C mutation, may account for the high penetrance and expressivity of essential hypertension in a Han Chinese family [19]. Moreover, the T7505C mutation was first described in a Han Chinese family with maternally transmitted non syndromic hearing impairment [20], structurally, the T7505C mutation was localized at the second base-pairing (10A-20U) on the D-stem of tRNA\textsuperscript{Ser(UCN)}. The phylogenetic analysis of this mutation and mtDNAs from the other 10 vertebrates revealed that the nucleotide A at the conventional position 10 in tRNA\textsuperscript{Ser(UCN)} was extremely evolutionarily conserved [21], ~65.0% reductions in the level of tRNA\textsuperscript{Ser(UCN)} were observed in the lymphoblastoid cell lines carrying this mutation [20]. Furthermore, the homoplasmic G15927A in the anticodon stem of tRNA\textsuperscript{Thr} was also described in a Han Chinese family with maternally inherited hearing loss [22]; later, this mutation was reported to be associated with coronary heart disease [23]. Northern blot analysis of the cell lines with the G15927A mutation revealed ~80.0% decrease in the steady-state level of tRNA\textsuperscript{Thr}, in addition, ~39.0% reduction in aminoacylated efficiency of tRNA\textsuperscript{Thr} was observed in mutant cells derived from the family members carrying the G15927A mutation. An increasing production of reactive oxygen species was observed in the mutant cells carrying the G15927A mutation. Therefore, we proposed that the G15927A mutation was a pathogenic mutation associated with lung cancer.

Based on these findings, we proposed that the molecular mechanism underlying these mt-tRNA mutations in the carcinogenesis of lung cancer may be as follows: first, these mutations disrupted the mt-tRNA secondary structure and subsequently resulted in failure in tRNA metabolism such as a CCA addition, posttranscriptional modification and aminoacylation [24]. Whatever the consequence may be, the expected net effect would be a decrease in mitochondrial protein synthesis. Defects in mitochondrial translation consequently leads to a respiratory phenotype and a decline in adenosine triphosphate (ATP) production below the threshold level required for normal cell function, thus, contributing to the tumorigenesis of lung cancer. In summary, this is the first report concerning the association between mt-tRNA mutations and lung cancer. The main limitation of this study was the sample size. Further studies including more samples are needed to verify the conclusions.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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