

Original Article

The role of mitochondrial tRNA mutations in lung cancer

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Abstract: Alternations in mitochondrial genome resulting in mitochondrial dysfunction have long been hypothesized to be involved in tumorigenesis. Mitochondrial tRNA (mt-tRNA) is known for its high frequencies of polymorphisms and mutations, however, the roles of these mutations and polymorphisms in lung cancer are among heated debates. To evaluate the possible roles of reported mt-tRNA mutations in lung cancer, we examine recent published paper concerning three mt-tRNA mutations with lung cancer: A7460G in tRNA^{Ser}(UCN) gene, G5563A in tRNA^{Trp} gene and A12172G in tRNA^{His} gene. We perform the phylogenetic approach to investigate the deleterious roles of these mutations in lung cancer, moreover, we use bioinformatics tool to predict the secondary structure of mt-tRNAs with and without these mutations. In addition, through the application of pathogenicity scoring system, we find that only the A12172G mutation is regarded as a pathogenic mutation, whereas other mutations may act as neutral polymorphisms in human population. Thus, our study provides the novel insight into the molecular pathogenesis of mt-tRNA mutations in lung cancer.

Keywords: mt-tRNA mutations, pathogenicity, lung cancer

Introduction

Lung cancer is a major public health problem worldwide. Nevertheless, in contrast to most Western countries, where lung cancer death rates are decreasing, lung cancer incidence rate is still increasing in China [1, 2]. The opposite trend of lung cancer incidence rate in China compared with that in Western countries might mainly be attributable to tobacco epidemic. Other identified risk factors for lung cancer in China include air pollution [environmental or occupational exposure to agents such as asbestos, nickel, chromium, and arsenic indoor air pollution from unventilated coal-fueled stoves and cooking fumes, especially for Chinese women] [3], and several genetic variants such as ATM, XPD, XRCC1 and OGG1 genes [4, 5].

However, most of these genetic variants occur at the nuclear genes, to date, the role of mitochondrial DNA (mtDNA) mutations in human lung cancer remain poorly understood. Mitochondria are ubiquitous organelles in eukaryotic cells whose primary role is to generate energy supplies in the form of ATP through oxidative phosphorylation [6]. The mammalian mitochondrial genome is a double-stranded cir-

cular DNA of ~16569 nucleotides. It contains 37 genes encoding 13 peptides for the oxidative phosphorylation apparatus, as well as 22 tRNAs and 2 rRNAs essential for protein synthesis within mitochondria.

In this study, we reassess the role of three reported mt-tRNA mutations: A7460G in tRNA^{Ser}(UCN) gene, G5563A in tRNA^{Trp} gene and A12172G in tRNA^{His} gene in clinical expression of lung cancer [7]. We first carry out the evolutionary conservation analysis of these mt-tRNA mutations; moreover, we perform RNA Fold Web Server to predict the minimum free energy (MFE) of mt-tRNAs with and without these mutations. We also use the pathogenicity scoring system to discern whether these mt-tRNA mutations contribute to the genetic susceptibility of lung cancer.

Materials and methods

Search strategy

We carry out a search in Pubmed Central and other public domains with the combination of the following key words “mitochondrial tRNA mutations, lung cancer” or “mitochondrial tRNA variants, lung cancer” to identify the case-con-

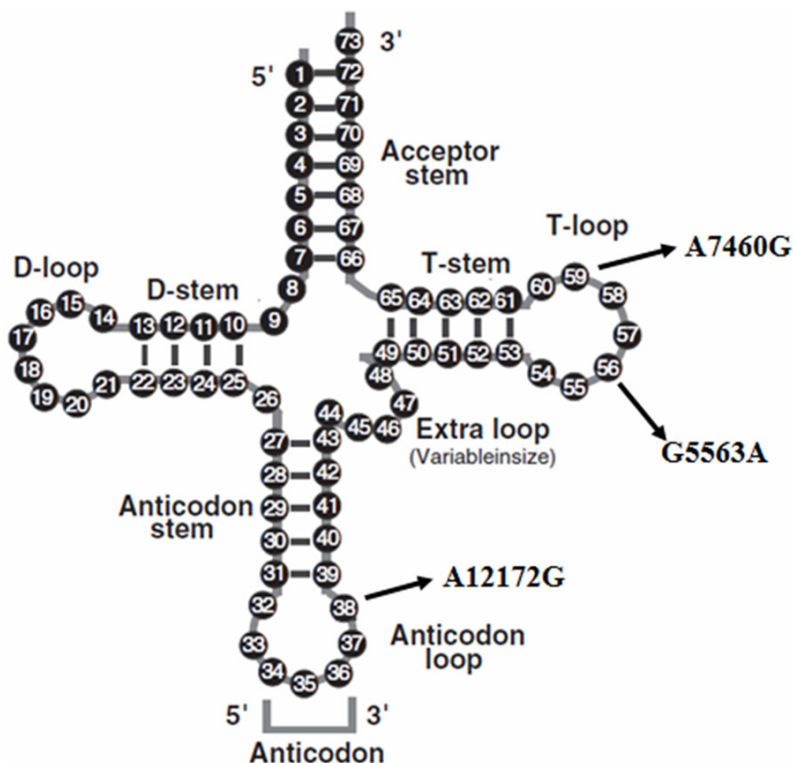


Figure 1. Cloverleaf structure of mt-tRNA with standard nucleotide numbering, the arrows indicate the positions of three mt-tRNA mutations: A7460G, G5563A and A12172G.

trol studies on the association between mt-tRNA mutations and lung cancer.

Phylogenetic analysis of the mt-tRNA mutations

With the purpose of understanding the possible role of mt-tRNA mutations in lung cancer, we perform evolutionary conservation analysis for the mt-tRNA mutations between different species. Briefly, 15 vertebrate's mtDNA sequences are used in the inter-specific analysis. The conservation index (CI) is then calculated by comparing the human nucleotide variants with other 14 vertebrates. The CI is defined as the percentage of species from the list of 14 different vertebrates that have the wild-type nucleotide at that position. Notably, the CI $\geq 70\%$ is considered as having the functional potential.

Prediction of the secondary structure of mt-tRNA with and without these mutations

To see whether the candidate mt-tRNA mutations alter the secondary structure of tRNAs,

we use RNA Fold Web Server to predict the MFE secondary structure of the wild type of mt-tRNA and the mutant carrying these mutations [8]. In addition to MFE folding, equilibrium base-pairing probabilities are calculated via John McCaskill's partition function (PF) algorithm [9].

Assigning pathogenicity to these tRNA mutations

McFarland's team [10] provided a program for assigning a pathogenicity score to mt-tRNA mutations, and their weighting scoring system was revised in 2011 [11]. According to that standard, a mt-tRNA mutation will be classified as "neutral polymorphism" if its score is less than 6 points, while the score of a candidate mutation is 7-10 points, it belonged to "possible pathogenic", whereas a mutation with the score more than 11 points, it is classified as "definitely pathogenic".

Results

Studies concerning the mt-tRNA mutations and lung cancer

We identify three mt-tRNA mutations that have been reported to be associated with lung cancer, the A7460G in mt-tRNA^{Ser (UCN)} gene; the G5563A in tRNA^{Trp} gene and the A12172G in tRNA^{His} gene [7].

Evolutionary conservation assessment of mt-tRNA mutations

Assessing pathogenicity of a nucleotide substitution in mt-tRNA gene involves evaluation of the evolutionary conservation of the base involved. As shown in **Figure 1**, the A7460G mutation occurs at position 59 in the T-loop of mt-tRNA^{Ser (UCN)} gene with a CI of 67%. Similarly, the CI of the G5563A (position 56) and A12172G (position 38) are 15% and 100%,

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Organism	Acc-stem		D-stem		D-loop		D-stem		Ac-stem		Anticid-loop		Ac-stem		V-region		T-stem		T-loop		T-stem		Acc-stem	
	1	8	10		22	26	27	32	38	39	44	49			61	66			73					
<i>Cavia porcellus</i>	GTAATTA	TA	GTTT	AACAA	AAAC	A	TTAGA	TTGTGAA	TCTAA	CAAT	AGAAG	ATTAACC	CTTCT	TATTTAC	C									
<i>Thryonomys swinderianus</i>	GTAATTA	TA	GTTT	AACAA	AAAC	A	TTAGA	CTGTGAA	TCTAA	CAAT	AAAAG	ATTATA	CTTTT	TAATTAC	C									
<i>Crocidura russula</i>	GTAGATA	TA	GTTT	AATAA	AAAC	A	TTAGA	TTGTGAA	TCTAA	TAAT	AGAAG	CITACAT	CTTCT	TATCTAC	C									
<i>Episoriculus fumidus</i>	GTAATAA	TA	GTTT	AACCA	AAAT	A	TTAGA	TTGTGAA	TCTAG	TGAT	AGAAG	ATAACAT	CTTCT	TATTTAC	C									
<i>Gorilla gorilla</i>	GTAATAA	TA	GTTT	AACCA	AAAC	A	TCAGA	TTGTGAA	TCTGA	TAAC	AGAGG	CTCACAA	CCCCT	TATTTAC	C									
<i>Pongo pygmaeus</i>	GTAATAA	TA	GTTT	AACCA	AAAC	A	TTAGA	TTGTGAA	TCTAA	TAAT	AGGGC	CCCACAA	CCCCT	TATTTAC	C									
<i>Homo sapiens</i>	GTAATAA	TA	GTTT	AACCA	AAAC	A	TCAGA	TTGTGAA	TCTGA	CAAC	AGAGG	CITACGA	CCCCT	TATTTAC	C									
<i>Nannospalax ehrenbergi</i>	GTAATAA	TA	GTTT	ACACA	AAAC	A	TTAGA	CTGTGAA	TCTAA	AAAT	AGAAA	ACACAT	TTTCT	TATTTAC	C									
<i>Talpa europaea</i>	GTAGATA	TA	GTTT	AACAA	AAAC	A	TTAGA	TTGTGAA	TCTAA	AAAT	AGGGT	ATCATA	CCCCT	TATCCAC	C									
<i>Bos taurus</i>	GTAATAA	TA	GTTT	AACAA	AAAC	A	TTAGA	TTGTGAA	TCTAA	CAAT	AGAAA	CCTATTAC	CTTCT	TATTTAC	C									
<i>Herpestes javanicus</i>	GTAATAA	TA	GTTT	AATATA	AAAC	T	TTAGG	TTGTGAA	CCTAA	CAAT	AGAAG	TTTGAAT	CTTCT	CATTTAC	C									
<i>Sus scrofa</i>	GTAGATA	TA	GTTT	AATAA	AAAC	C	CTAGA	TTGTGAA	TCTAG	TAAT	AGAAA	ATTAAAT	ATTCT	TATCTAC	C									
<i>Lemur catta</i>	GTAATAA	TA	GTTT	AAAA	AAAC	A	CTAGA	TTGTGAA	TCCAG	AAAT	AGAAG	CTCAAAC	CTTCT	TATTTAC	C									
<i>Zaglossus bruijnii</i>	GTAGATA	TA	GTTT	AATAA	TAAC	A	TTAGA	TTGTGAA	TCTAA	AAAT	AGAAG	TTTAATT	CTTCT	TATCTAC	C									
<i>Colobus guereza</i>	GTAATAA	TA	ATTT	AACCA	AAAT	A	CTAGA	TTGTGAA	CCTAG	ACAT	AGAAG	CITACCG	CTTCT	TATTTAC	C									

Figure 2. Alignment of tRNA^{His} gene from different species, arrow indicates the position 38, corresponding to the A12172G mutation.

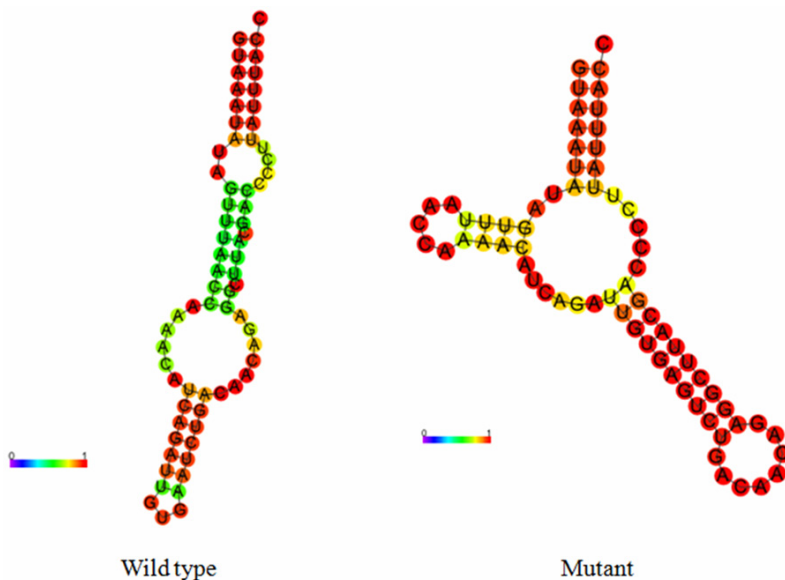


Figure 3. Prediction of the MFE structure of tRNA^{His} gene with and without the A12172G mutation.

respectively, indicated that the A12172G mutation is conserved between different species (Figure 2).

MFE prediction

We further use RNA Fold Web Server to predict the MFE change between the wild type of tRNAs and the mutant. As shown in Figure 3, only the A12172G mutation alters the secondary structure of tRNA^{His}, and caused the thermodynamic change between the wild type and the mutant, whereas other mutations do not have such effects (Table 1).

Pathogenicity scoring system for these tRNA mutations

According to the revised pathogenicity scoring system for mt-tRNA mutations [11], we classify the A12172G mutation as “possibly pathogenic” with a total score of 9 points (Table 2), whereas the scores of the A7460G and G5563A mutations are 3 and 2 points, respectively, suggests that these mutations belong to “neutral polymorphisms”.

Discussion

Mt-tRNA point mutations are being increasingly recognized as important causes of disease, with novel pathogenic changes being reported across all the mt-tRNAs. Such mutations can result in transcriptional and translational defects and consequently mitochondrial respiratory chain dysfunction [12], and are associated with clinical features as diverse as cardiomyopathy, chronic progressive external ophthalmoplegia (CPEO), and cancer [13]. However, due to its high mutation rate, mt-tRNA mutations have been reported increasingly, and a poor genotype to phenotype correlation is common, as in the case of mt-tRNA^{Phe} C628T vari-

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Table 1. Characterization of mt-tRNA mutations

Mutation	Location	Homoplasmy/Heteroplasmy	MFE (Wild type) kcal/mol	MFE (Mutant) kcal/mol	CI
A7460G	T-loop	Homoplasmy	-20.54	-21.08	67%
G5563A	T-loop	Homoplasmy	-10.82	-10.82	15%
A12172G	Anticodon Stem	Heteroplasmy	-11.16	-14.62	100%

Table 2. The pathogenicity scoring system for the A12172G mutation

Scoring criteria	A12172G mutation	Score/20	Classification
More than one independent report	Yes	2	
Evolutionary conservation of the base pair	no change	2	
Variant heteroplasmy	Yes	2	≤6 points: neutral polymorphisms;
Segregation of the mutation with disease	Yes	2	7~10 points: possibly pathogenic;
Histochemical evidence of mitochondrial disease	No evidence	0	≥11 points: definitely pathogenic
Biochemical defect in complex I, III or IV	No	0	
Evidence of mutation segregation with biochemical defect from single-fiber studies	No	0	
Mutant mt-tRNA steady-state level or evidence of pathogenicity in trans-mitochondrial cybrid studies	Weak evidence	1	
Maximum score		9	Possibly pathogenic

ant [14]. Distinguishing the polymorphisms and mutations in mt-tRNAs is important, since failure to do so will inevitably lead to poor diagnostic and genetic advice.

In this study, we analysis the possible role of three mt-tRNA mutations: A7460G, G5563A and A12172G in clinical expression of lung cancer through the application of bioinformatics and phylogenetic conservation analysis. We notice that the homoplasmic G5563A mutation is regarded as a neutral polymorphism in human mitochondrial disease studies [15-17], which is consistent with our results, as this variant lacks the evolutionary conservation and does not alter the secondary structure of tRNA^{Trp} (Table 1). In addition, the A7460G mutation occurs at position 59 in the T-loop of tRNA^{Ser(UCN)}, nevertheless, this mutation has not been reported in Mitomap or mtDB, suggests that it is a rare variant with allele frequencies <0.5%. By contrast, the A12172G mutation is reported to increase the penetrance and expressivity of LHON-associated ND4 G11778A mutation [18], and it has been found in patients with Lactic acidosis [19]. In the case of the A12172G mutation, investigation of phylogenetic conservation supports that this mutation may be polymorphic, because it belongs to haplogroup D4b1b1a [20], however, RNA Fold

results show that the A12172G mutation causes the MFE change of tRNA^{His}, indicates that it may cause pathogenicity by a mean of disrupting the formation of the anticodon stem, which may in turn decrease the efficiency of codon-anticodon recognition and aminoacylation.

It should be noted that each cell contains hundreds to thousands of copies of mtDNA. Once a pathogenic mutation arises in mtDNA, a certain population of that pathogenic mtDNA will coexist with wild type mtDNA, a condition known as heteroplasmy. When the frequency of the pathogenic mtDNA in a cell increases and exceeds a threshold, a clinical phenotype appears. This observation highlights the importance of the heteroplasmic mtDNA mutations in clinical diseases, suggests that the heteroplasmic A12172G mutation may be involved in the pathogenesis of lung cancer.

Based on these observations, we proposed that the molecular mechanism underlying the A12172G mutation in the tumorigenesis of lung cancer may be as follows: first, the mutation itself disrupts the mt-tRNA secondary structure and subsequently results a failure in tRNA metabolism such as the CCA addition, post-transcriptional modification and aminoacylation [21]. In particular, post-transcriptional

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modifications may alter the specificity or stability of the tRNA or change its affinity for its codon. 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 ATP production below the threshold level required for normal cell function [22], thus, contributes to the tumorigenesis of lung cancer. Taken together, this is the first time that our study provides the direct evidence for the lung cancer associated mt-tRNA A12172G mutation.

Disclosure of conflict of interest

None.

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