Mitochondrial D310 instability in Chinese lung cancer patients

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Abstract

To characterize the somatic mutation spectrum of mitochondrial DNA at D310 in Chinese lung cancer patients and evaluate its potential significance in Chinese lung cancer diagnosis, in this study, 237 samples, including lung tumor, adjacent normal tissue and blood samples of 79 lung cancer patients were analyzed. By comparing sequences of D310 between lung cancer tissues, adjacent normal tissue and blood samples, the somatic mutations at D310 were detected in 17.72% (14/79) of Chinese lung cancer patients; this implied that somatic mutations at D310 could be served as valuable biomarker for diagnostic of Chinese lung cancer. Further analyses indicated that deletion and heterogeneity were the predominant characters for somatic mutations detected at D310 of Chinese lung cancer patients.

Keywords

Chinese, D310 mutation, lung cancer, mitochondrial DNA

Introduction

Lung cancer was one of the major public health challenges in the world, accounting for around 30% of all cancer related death (Cao et al., 2011, Ferlay et al., 2010). And the mortality rate of lung cancer in China was approximately 400,000 patient deaths annually (Yang et al., 2009). However, it was still unclear for the mechanism of lung cancer carcinogenesis. Mitochondria plays important role on generating ATP, producing reactive oxygen species, and initiating and executing apoptosis. Thus, recent investigations have pinpointed the potential role of mitochondrial DNA (mtDNA) changes in carcinogenesis and mtDNA mutations in tumorigenesis have received much attention (Baysal, 2006; Chatterjee et al., 2006; Pereira et al., 2012; Salas et al., 2005; Schon et al., 2012; Yu, 2012 Yu et al., 2013). And more evidences have revealed the somatic mutations may be involved in carcinogenesis and tumor progression (Li & Hong, 2012; Yu, 2012).

Human mtDNA genome includes a displacement region (D-loop) and coding region segments (Anderson et al., 1981; Andrews et al., 1999). The D-loop locates at nucleotide positions between 16,024 and 576, it is a non-coding region with essential elements on regulating mtDNA duplication and transcription (Li et al., 2012). MtDNA genome has a more than 10-fold of mutation rate than that of nuclear genes for inefficient DNA repair mechanism, lacking of protectase-free survival, later onset age (Tseng et al., 2006). The cive histones and higher level of reactive oxygen species (ROS) circumstance (Wallace et al., 1999). The high incidence of somatic mtDNA mutations were identified in nearly all types of cancerous tissues (Hung et al., 2010; Li & Hong, 2012; Liu et al., 2001, 2012; Navaglia et al., 2006; Van et al., 2007; Wang et al., 2007; Yu, 2012; Yu et al., 2013). Thus, many researchers claimed that somatic mtDNA mutation could be used as biomarker in early cancer diagnosis (Arasaradnam et al., 2006; Cai et al., 2011; Fliss et al., 2000; Sui et al., 2006; Tseng et al., 2006). This implied that the somatic mutations can serve as a marker for early tumor diagnostic, especially the a poly-C tract located between 303 and 315 nucleotides, known as D310, for its higher frequency in tumors (Arasaradnam et al., 2006; Cai et al., 2011; Sui et al., 2006). Patients with poor comprehensive knowledge is indispensable for us to understand tumorigenesis and develop personalized therapies (Wang et al., 2013). However, there was still lacking of genetic dataset for the somatic mutations at D310 in Chinese lung cancer patients, thus, in this study, to characterize the spectrum of mtDNA somatic mutation at D310 in Chinese lung cancer patient and evaluate its potential significance in Chinese lung cancer diagnosis, 237 samples, including lung tumor, adjacent normal tissue and blood samples of 79 lung cancer patients were analyzed.

Materials and methods

Tissue specimens

Samples of 79 lung cancer patients including the primary lung cancerous tissue, corresponding paracancerous normal tissue and distant normal tissue of each patient were collected who received treatment at the first affiliated hospital of Kunming medical university and The second people’s hospital of Yunnan province between 2011 and 2012. The samples were handled as described in our previous work (Fang et al., 2013). All procedures were supervised and approved by human tissue research committee of our hospital, and informed consents were obtained from all participants.
DNA extraction, PCR amplification and sequence analysis

The genomic DNA was extracted with the standard phenol/chloroform method, and stored at $-20^\circ$C for future usage. The forward primer, 5'-GGTCTATCACCTATTA ACCAC-3' (nucleotides 8–29) and reverse primers, 5'-TGAGAGGTAAAGCT ACATA AACT G-3' (nucleotides 598–575) were used for amplifying fragment with D310 polymorphisms. The PCR products were purified, sequenced and analyzed as described in our previous work (Fang et al., 2013).

Verification of mtDNA mutations

The purified PCR product including the somatic mutation from the cancerous tissue and blood were transferred to the pUC18 vector. The clones were selected and sequenced directly with the forward and reverse vector primers, and the sequences were compared with the revised Cambridge reference sequence (rCRS; Andrews et al., 1999).

Results and Discussion

To characterize the spectrum of mtDNA somatic mutation at D310 in Chinese lung cancer patient and evaluate its potential significance in Chinese lung cancer diagnosis, we compared the sequences covering the D310 of 237 samples from 79 unrelated lung cancer patients from Yunnan Province of China. The difference of mtDNA sequences between tumor and matched normal tissues from the same patient was defined as a somatic mutation. The fragment of D310 with the consistent cytosine repeats has the concord signal as shown in Figure 1(a); in verse, the tissue with inconsistent cytosine repeats has the overlapping signal as listed in Figure 1(b). As shown in Table S1 (supporting material online), totally 14 somatic mutations at D310 were detected among 79 Chinese lung cancer patients.

To consolidate the authenticity of the somatic mutations, genome DNA of different tissue for the same individual with the somatic mutation was re-extracted, independent PCR was amplified with the fragment including the cytosine repeats at D310, and the purified PCR products were transferred into the pUC18 vector, and the clones were amplified and sequenced with the common forward and reverse primers of the pUC18 vector. As shown in Figure 2, the somatic mutations of the 14 Chinese lung cancer patients were verified, which accounted 17.72% (14/79) of the investigated patients, however, the frequency of the somatic mutations detected at D310 fragment in Chinese lung cancer patients was lower than that of breast cancer (Alhomidi et al., 2013; Xu et al., 2012), especially that of squamous cell carcinoma in situ of the head and neck (63.5%, 8/13) (Ha et al., 2002), previous reported lung cancers (70%, 10/14) (Fliss et al., 2000), and the different sample size was the main reason causing the different frequency of somatic mutations detected at D310. And the susceptible to oxidative damage and electrophilic attack owing to the poor DNA repair system in mitochondria can accumulate mutations at D310 (Mambo et al., 2003). Together with the associations between somatic mutations detected at D310 and poor disease-free survival, a late onset age, estrogen and progesterone-negative cancers (Tseng et al., 2006), and the higher frequency of somatic mutation rates in D310 fragment of Chinese lung cancer patients, which implied that detecting the somatic mutations at D310 fragment could be used as potential valuable molecular marker for diagnostic of Chinese lung cancer.

Further, we analyzed the patterns of 14 somatic mutations at D310 fragment. Our results indicated that deletion formed the predominant form of the somatic mutations at D310 fragment, which account for 78.57% (11/14) of the patients with somatic mutations. In addition, 21.43% (3/14) of which was insertion. As revealed in health individuals, the polymorphisms at D310 sequence was consistent of cytosine repeats in the same individual (Andrews et al., 1999), it ranges from seven to nine of cytosine repeats among different individuals (Ha et al., 2002). And our results indicated that all the somatic mutations were detected by comparing the sequences of lung cancer tissue and blood (distant normal tissue), and the polymorphisms at D310 sequence from the blood have the consistent of variations of cytosine repeats for the same individual, which indicated that comparing the sequences of the primary lung cancerous tissue and blood (distant normal tissue) can identify the somatic mutations efficiently, rather than comparing the sequences of the primary lung cancerous tissue, corresponding paracancerous normal tissue and distant normal tissue of each patient. However, the somatic mutations at D310 were inconsistent of the cytosine repeats for the tumor tissue, most of which were at the heterogeneity status, which indicated that the heterogeneity was another character of the somatic mutations for Chinese lung cancer patients.

In summary, our results indicated that the somatic mutations at D310 accounted 17.72% of all the investigated Chinese lung cancer patients, which implied that detecting the somatic mutations at D310 could be served as valuable biomarker for diagnostic of Chinese lung cancer; and detecting the differences of D310 repeats between the primary lung cancerous tissue and distant normal tissue were an efficient method for checking the somatic mutations. Deletion formed the major form of the somatic mutations and heterogeneity was another character of D310 repeats for the tumor tissue.

Figure 1. Concordant (A) and inconsistent (B) sequences results between tumor and distant normal tissue.
Figure 2. mtDNA somatic mutations detected at D310 fragment in Chinese lung cancer patients. Sequencing results of the lung cancer tissue and distant normal tissue from the same patient. And the positions were marked with the red arrows. The mutations were recorded by comparing with rCRS.

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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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