Legal Medicine 30 (2018) 38–41

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Forensic evaluation of STR typing reliability in lung cancer

Peng Zhang[a,1], Ying Zhu[a,1], Yongguo Li[a,b], Shisheng Zhu[c], Ruoxiang Ma[a,b], Minzhu Zhao[a,b], Jianbo Li[a,*]

[a] Department of Forensic Medicine, Faculty of Basic Medical Sciences, Chongqing Medical University, Chongqing 400016, China
[b] Chongqing Engineering Research Center for Criminal Investigation Technology, Chongqing 400016, China
[c] Faculty of Medical Technology, Chongqing Medical and Pharmaceutical College, Chongqing 401331, China

ARTICLE INFO

Keywords:
Lung cancer
Short tandem repeats
Forensic science
Microsatellite instability

ABSTRACT

Short tandem repeats (STR) analysis is the gold standard method in the forensics field for personal identification and paternity testing. In cancerous tissues, STR markers are gaining attention, with some studies showing increased instability. Lung cancer, which is one of the most common malignancies, has become the most lethal among all cancers. In certain situations, lung cancer tissues may be the only resource available for forensic analysis. Therefore, evaluating the reliability of STR markers in lung cancer tissues is required to avoid false exclusions. In this study, 75 lung cancer tissue samples were examined to evaluate the reliability of various STR markers. Out of the 75 examined samples, 24 of the cancerous samples (32%) showed genetic alterations on at least one STR loci, totaling 55 times. The most common type of STR variation was a partial loss of heterozygosity, with the D5S818 loci having the highest variation frequency and no alterations detected on the D2S441 and Penta E loci. Moreover, STR variation frequencies were shown to increase with an increased patient age and increased clinical and pathological characteristics, thus an older patient with an advanced stage of progression exhibited a higher variation frequency. Overall, this study provides forensic scientists with further insight into STR analysis relating to lung cancer tissue.

1. Introduction

Short tandem repeats (STRs), also known as microsatellites, are genomic DNA sequences that consist of repeating units (2–6 bp) [1]. In eukaryotic genomes, STRs are extensively distributed in non-coding regions and characterized by co-dominant inheritance, high levels of polymorphisms, high reliability and good reproducibility; thus making them effective for forensic analysis [1,2]. As second generation genetic marks, STRs are broadly used in forensics for personal identification and paternity testing [2–4]. In forensic medicine, STRs are selected based on a specific association with known disease markers. However, a growing number of studies examining human cancers and inherited diseases have found that STRs can be unstable [2]. In cancerous tissues, microsatellite loci stability can be affected by factors such as defects in repair mechanisms, frequent mutation incidences or chromosomal abnormalities [5,6].

Recently, STR reliability in human cancer tissues has gained more and more attention from forensic scientists. This change is largely attributed to the increased morbidity of various cancers and rising tension between doctors and patients, which can inevitably lead to forensic analysis in some special cases [7,8]. Recently, Filoglu et al. demonstrated that genetic instabilities could be detected in leukemia patient blood samples [2]. In some particular situations, a tumor tissue may be the only source of biological material available for a forensic DNA profiling investigation, such as when a patient suspects a sample mix up or if a putative father was dead and no other tissue samples were left or other special cases [2,7].

In the last decade, lung cancer morbidity and mortality rates have rapidly increased [9]. According to a recent epidemiological study, more than 1.8 million people worldwide are newly diagnosed with lung cancer each year, thus account for 12.9% of all cancer patients and making it the most lethal cancer [9,10]. Lung cancer tissues, in particular situations, are the only sample available for paternity testing or individual identification analysis. Therefore, it is extremely important to evaluate the reliability of lung cancer STRs to avoid obtaining a false exclusion result, especially when a complete loss of one allele or loss of heterozygosity (LOH) and/or a new allele has occurred in the tissue. In one study examining 24 lung carcinoma tissue samples, most samples showed microsatellite instability (MSI) in at least one STR loci, with LOH detected in one small-cell carcinoma sample [11]. However, this

* Corresponding author at: Yixueyuan Road 1#, Yuzhongqu, Chongqing 400016, China.
E-mail address: ljb2032008@outlook.com (J. Li).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.legalmed.2017.11.004
Received 22 June 2017; Received in revised form 26 August 2017; Accepted 10 November 2017
Available online 11 November 2017
1344-6223/

study had some limitations, like a small sample size and a lack of correlation analysis between STR variation frequency and patient clinical and pathological characteristics. Furthermore, other recent studies have suggested that MSI and LOH are associated with tumor occurrence and development, with LOH levels closely correlated with tumor progression [2,5].

In this study, STR typing was performed in 75 patients, with blood samples and paired lung cancer tissue and normal adjacent normal tissue examined. The aim of this study was to evaluate the reliability of various STR markers in malignant lung cancer tissue as a source for forensic analysis. Overall, this study provides further insight into the incidence and patterns of lung cancer STR alterations which can be applied during a forensic analysis to reduce cases of false exclusion and can aid in lung cancer detection and treatment.

2. Materials and methods

2.1. Lung cancer sample collection and histopathologic examination

Analysis was performed on 75 patient samples (33 squamous cell carcinomas and 42 adenocarcinoma), with lung cancer tissue, corresponding tumor-adjacent normal lung tissue, and a blood sample examined for each patient. All of the samples were obtained from the First Affiliated Hospital of Chongqing Medical University (Chongqing, China) along with detailed clinical and pathological information, to include gender, age at surgery/diagnosis, histological type and TNM scoring based on the AJCC system. Informed consent was obtained from each patient and the study was approved by the Ethical Committee of Chongqing Medical University. All patients in this study had not received radiotherapy, chemotherapy or other cancer treatments prior to surgery. After samples were collected, carcinoma and adjacent normal tissues were selected via microscopy as previously described to avoid contamination [11] and pathologic analysis was performed by two pathologists independently (Fig. S1).

2.2. DNA extraction, PCR amplification and STR typing

DNA was extracted from the paired carcinoma tissue, adjacent normal tissue and blood samples using a Genomic DNA kit (TIANGEN Biotech, Inc., Shanghai, China) according to the manufacturer's instructions. The obtained DNA was then quantified by fluorescent quantitative PCR using a Qubit® dsDNA HS Assay kit (ThermoFisher, Inc., Waltham, USA). Twenty autosomal STR loci (D19S433, D5S818, D21S11, D18S51, D6S1043, D3S1358, D13S317, D7S820, D16S539, vWA, CSF1PO, Penta D, D2S441, D8S1179, TPOX, Penta E, TH01, D12S391, D2S1338 and FGA) and the AMEL loci were amplified using a Microreader™ 21 Direct ID System kit (Beijing Microread Genetics Co., Ltd, Beijing, China) in a GeneAmp 9700 thermal cycler according to the manufacturer's instructions. Each PCR reaction mixture contained 7 μl Buffer B, 2 μl Primer Mix, 0.2 μl DNA Polymerase II and 1 μl (0.45–1.1 ng/μl) extracted DNA. The PCR reaction conditions were as follows: 96 °C for 2 min, followed by 27 cycles at 94 °C for 5 s and 60 °C for 70 s, then 72 °C for 20 min and a 15 °C hold. The amplicons were then processed by capillary electrophoresis using a 3130 Genetic Analyzer (AB SCIEX, Massachusetts, USA) and STR typing was performed using Gene Mapper ID software v3.2 (AB SCIEX, Massachusetts, USA). Samples with a genotype difference between the cancer tissue and the corresponding adjacent normal tissue or blood sample were repeatedly typed to ensure reproducibility and reliability of the results. All of the STR variations and variant types were confirmed based on medical opinions from two independent forensic scientists, with a third expert engaged if a discrepancy arose.

2.3. STR typing data analysis

Four types of genetic alterations, which included complete LOH, partial LOH, new alleles and additional alleles, were statistically analyzed for the 21 STR loci in all 3 of the paired samples for each patient. A new allele was defined as the disappearance of an original allele, with it being replaced by a new allele; such as "8, 11" alleles in the normal tissue mutating into "8, 12" in the cancer tissue. An additional allele is defined as an additional allele appearing with the original alleles in the cancer tissues; such as "8, 11" alleles in the normal tissue mutating into "8, 10, 11" in the cancer tissue. Partial LOH was considered at an allele peak height ratio > 0.6 when comparing the cancerous and corresponding normal tissue [12].

Chi-Square and Cochran-Armitage tests were used for statistical analysis to explore the relationships between STR variation and gender, age at surgery, histological type and TNM staging of the patients.

3. Results

3.1. STR variation frequencies and types

All of the STR loci in all 3 paired samples for each patient were successfully typed. No genotype differences were detected between the blood and tumor-adjacent normal lung tissue samples for the 75 patients that were examined. However, STR variations were noted 55 times within 24 of the lung cancer tissue samples (32% mutation rate). Among the identified STR variants within the cancerous tissues, 10 were LOH, 35 were partial LOH and 10 included additional alleles, with no new alleles detected (Fig. 1). Thus, the most common STR variant type was partial LOH (63.64%). These results demonstrate that different types of genetic alterations can occur in multiple STR loci and at different frequencies in lung cancer tissues (Table 1).

3.2. Correlative analysis of STR variations and patient demographic characteristics

Chi-square tests were used to determine associations between TR variants and a patient's gender, age at surgery and histological type. The results showed that no significant correlation existed between the STR variants and patient gender or histological type, but a significant correlation was found between the STR variants and the patient's age at surgery/diagnosis (Table 2). Cochran-Armitage trend tests were performed to determine the association between STR variants and patient TNM stage and showed that STR variation frequencies were higher in patients at an advanced TNM stage relative to those at an earlier stage (Table 2). Furthermore, older patients at an advanced TNM stage exhibited the highest frequencies of STR variations (Table 2).

4. Discussion

In recent years, lung cancer incidences and mortality rates are increasing worldwide, thus making it a serious health issue [9,10]. One previous study suggested that MSI can be detected in lung cancer tissues, but that study was limited by a small sample size and clinical data [11]. In this study, 75 patient samples were examined, with lung cancer tissue, tumor-adjacent normal lung tissue and a blood sample examined from each patient, to evaluate the reliability of selected STR markers. In 24 of the cancerous tissue samples, genetic alterations on at least one STR loci were noted 55 times. The types of genetic alteration included LOH (10 times), partial LOH (35 times) and additional alleles (10 times), with the partial LOH present at the highest frequency.

Among the 21 examined loci, the most frequent alterations occurred at D5S818 and D3S1358, which was consistent with a previous study [10]. In previous studies examining non-small cell lung carcinomas, LOH and MSI could be detected in chromosomal arms 1p, 2p, 2q, 3p, 4q, 5q, 6p, 6q, 7q, 7p, 8p, 9p, 10q, 11p, 13p, 13q, 17p, 18q, 19q, 21q and 22q, and certain LOHs and MSIs appeared to be associated with processes such as carcinogenesis, clinicopathological parameters and prognosis [13,14]. Herein, the genetic alterations detected at STR loci