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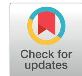
Comparison of genetic profiles among primary lung tumor, metastatic lymph nodes and circulating tumor DNA in treatment-naïve advanced non-squamous non-small cell lung cancer patients



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ABSTRACT

Objectives: Genetic profiles of primary and metastatic lung tumor have been investigated by previous studies. However, whether they can be replaced by each other to guide treatment remains controversial. Moreover, it is unclear that whether genetic profiles of plasma can reflect genetic divergence between primary and metastatic lesions.
Materials and methods: In this prospective study, we collected 35 pairs of matched primary tumor tissue, metastatic lymph nodes and plasma from treatment-naïve patients with advanced non-squamous non-small cell lung cancer (NSCLC) and applied to capture-based sequencing using a panel consisting 56 NSCLC-related genes to interrogate the heterogeneity and similarity among the 3 sites.
Results: We observed 62.0% (67/108) by-variant concordance rate among primary tumor, metastatic lymph nodes and plasma as well as 76.4% (81/106) by-variant concordance rate between primary tumor and metastatic lymph nodes. When the analysis restricted to driver genes, we achieved 60.9% (28/46) and 77.3% (34/44) concordance, respectively. Furthermore, there is no statistically significant difference in progression-free survival (PFS) of 17 patients who used matched targeted therapy between patients having 100% concordance rate between primary tumor and metastatic lymph nodes and patients having partially matched mutational profiles.
Conclusion: Collectively, our study revealed a similar genetic profile shared between primary tumor and metastatic lymph nodes. The limited discordance observed can be partially reflected by plasma. Sequencing results obtained from either site can be utilized for providing treatment guidance.

1. Introduction

Lung cancer is the leading cause of cancer-related death worldwide [1,2]. Targeted therapies, revolutionized the treatment of non-small cell lung cancer (NSCLC), have substantially improve the outcomes of a subpopulation of patients [3–5]. However, responses are often transient and there are still a significant percentage of patients who do not respond to treatment despite the fact of harboring targeted mutation [6]. Such phenomena can be partly attributed to the expansion of drug resistance clones, which have different genetic profiles, resulting in tumor heterogeneity [7], which imposes serious challenges on the development of therapeutic agents. Therefore, understanding tumor heterogeneity is critically important to the development of targeted therapies.

Metastasis, the main cause of death in individuals with cancer, is often depicted as a multistage process in which malignant cells spread from the tumor of origin to distant organs via blood circulation [8]. The prevailing model of metastasis holds that only a small subset of cells has the ability to metastasize, thus resulting in distinct molecular signature comparing to the primary tumor [9,10]. In contrast, other studies have reported evidence supporting the notion that genetic changes favoring

Abbreviations: AF, allelic fractions; ctDNA, circulating tumor DNA; cfDNA, circulating cell-free DNA; CNV, copy number variation; LOF, loss of function; maxAF, maximal AF; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; PFS, progression-free survival

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metastasis is an early event in tumorigenesis [11]. Distinct genotypes and phenotypes are referred to as tumor heterogeneity, which can occur within a primary tumor and its metastasis or between tumors of the same histopathological subtype [12]. Studies have shown the impact of tumor heterogeneity on addressing resistance mechanisms [13], treatment decisions and accurate diagnosis [14].

Although, currently the gold standard for obtaining mutation profile is still from tissue biopsy, either from the primary tumor or from a single metastatic lesion, the potential of probing genetic profile of solid tumors through blood draw—liquid biopsy has been increasingly acknowledged and routinely performed in clinical settings, especially in patients with advanced disease when tissue biopsy is not feasible [15,16]. The majority of circulating tumor DNA (ctDNA), composed of small fragments of nucleic acid, is released from apoptotic or necrotic tumor cells, thus reflecting genetic profiles of solid tumors [17]. The bias generated by tumor biopsy, which is a temporal and spatial snapshot of a tumor, to some extent can be overcome by liquid biopsy [18]. Numerous studies have demonstrated a high concordance between mutation profiles obtained through ctDNA and tumor biopsy [19].

Currently, in the management of NSCLC, genetic profiles obtained from primary tumor, metastatic lymph nodes or peripheral blood are all used for guiding treatment in clinical practice. However, whether primary tumor and metastatic lymph nodes can be replaced by each other to guide treatment remains controversial. Moreover, whether genetic profiles of plasma ctDNA can reflect the spatial heterogeneity between primary and metastatic lesions is unclear. This is the first prospective study designed to evaluate the mutational profiles of matched primary tumor, metastatic lymph nodes and peripheral blood using capture-based ultra-deep targeted sequencing. This cohort consisted of 35 treatment-naïve patients with advanced NSCLC.

2. Materials and methods

2.1. Patient selection

From May 2015 to July 2016, patients meeting the following inclusion criteria from Shanghai Chest Hospital were enrolled in this study. Inclusion criteria: 1) Treatment-naïve patients suspected to have advanced (IIIA-IV) non-squamous NSCLC according to the 7th edition of the TNM classification. 2) Between the age of 18–80 years old. 3) Chest imaging demonstrating primary lung tumor and at least one metastatic lymph node that can be sampled. 4) Not suitable for surgery as first-line treatment evaluated by multidisciplinary team. Exclusion criteria: 1) Patients suspected to have small cell lung cancer or squamous cell carcinoma. Specific guidelines included: patients who were heavy-smokers, having symptom of blood sputum, having a very high serum neuron-specific enolase (NSE) and squamous cell carcinoma antigen (SCCAg) level, chest imaging showing a central mass or a huge mass, chest imaging showing a mass with thick-walled cavity or matting of extensive lymph nodes. Patients were suspected to have small cell lung cancer or squamous cell carcinoma when these specific guidelines were considered together. 2) Received blood transfusion within one month. The study was approved by the local ethics committee (No.KS1506) and registered under ClinicalTrials.gov (NCT02416726). All patients participating in the study provided written informed consent.

2.2. Plasma DNA extraction

Ten ml of whole blood was collected and centrifuged at 2000 g for 10 min at +4 °C within 2 h after collection. Supernatant was transferred to a fresh 15 ml centrifuge tube without disturbing the buffy coat layer and subjected to an additional centrifugation for 10 min at 16,000*g* at +4 °C. The supernatant was again transferred to a new tube. The plasma was stored at −80 °C until further analysis. Circulating cell-free DNA (cfDNA) was extracted from plasma samples using the QIAamp Circulating Nucleic Acid kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Quantification of cfDNA was performed using the Qubit 2.0 Fluorometer with the dsDNA HS assay kits (Life Technologies, Carlsbad, CA, USA).

2.3. Tissue samples collection and DNA extraction

Tissue samples were obtained from small biopsies. Techniques used to provide lymph nodes samples included endobronchial ultrasound guided transbronchial needle aspiration and ultrasound-guided core needle biopsy of superficial lymph nodes. Samples with non-squamous NSCLC in both primary and metastatic lymph node tissues were enrolled in the study. All samples had tumor content greater than 20%. Tissue DNA was extracted using QIAamp DNA FFPE tissue kit (Qiagen) according to manufacturer's instructions. DNA concentration was measured using Qubit dsDNA assay (Life Technologies).

2.4. Next-generation sequencing (NGS) library preparation

DNA shearing was performed using Covaris M220, followed by end repair, phosphorylation and adaptor ligation. Fragments of size 200–400 bp were selected using Agencourt AMPure beads (Beckman Coulter, Brea, CA, USA) followed by hybridization with capture probes baits, hybrid selection with magnetic beads and polymerase chain reaction (PCR) amplification. A bioanalyzer high-sensitivity DNA assay was performed to assess the quality and size of the fragments. 50 ng of DNA was used for library construction. Twelve PCR cycles were used for library amplification. The indexed samples were sequenced on Nextseq500 sequencer (Illumina, Inc., San Diego, CA, USA) with pair-end reads (read length 150 bp).

2.5. Panel description

The panel used in this study was manufactured by Burning Rock Biotech, Guangzhou China. It covers whole exons and selected introns of 56 lung cancer related genes, spanning 330 kb of human genome, which were listed in the Supplemental Table 1.

2.6. Sequencing data analysis

The sequencing data in the FASTQ format were mapped to the human genome (hg19) using BWA aligner 0.7.10. Local alignment optimization, variant calling and annotation were performed using GATK 3.2, MuTect, and VarScan, respectively. DNA translocation analysis was performed using Factera 1.4.3, described previously [20]. Copy number variation (CNV) was detected by in-house analysis scripts based on depth of coverage data of capture intervals. Coverage data was firstly corrected against sequencing bias resulting from GC content and probe design. The average coverage of all captured regions was utilized to normalize the coverage of different samples to comparable scales. Copy number was calculated based on the ratio between depth of coverage in tumor samples and average coverage of an adequate number ($n > 50$) of samples without CNV as references as to each capture interval. Gene CNV is called if the coverage data of the gene region was quantitatively and statistically significantly different from its reference control. Following criteria should be met for CNV to be called: 1) the coverage of more than 60% capture intervals of the genes should be significantly different from the reference. The significance was evaluated by z-test comparing coverage of each capture interval to the mean coverage of the interval in all control samples ($p < 5e\text{-}3$ for hotspot genes and $p < 1e\text{-}3$ for others); 2) copy number should reach the minimum threshold as to gain and loss (as to copy number gain, $CN > 2.25$ for hotspot genes and $CN > 2.5$ for others; as to copy number loss, $CN < 1.75$ for hotspot genes and $CN < 1.5$ for others). By-variant concordance rate is defined as the same variant detected in different

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