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Case Report

Somatic mutation in synchronous primary adenocarcinomas of the left lung: a case report

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Abstract: Multiple lung adenocarcinomas (AC) are uncommon. We herein report a case of multiple AC. A 62-year-old Chinese woman was admitted to our hospital because of her chest enhanced computed tomography (ECT) finding. Her ECT revealed a suspected lung cancer in the left lower lung and ill-defined weak ground-glass opacity in the left upper lobe. Upon operation, however, both were pathologically diagnosed as AC, an invasive one and an in-situ one, respectively. Only the invasive AC presented epidermal growth factor receptor (EGFR) mutation, while anaplastic lymphoma kinase (ALK) rearrangement was detected in none of them. To not miss rare invasion-related mutations, the whole exome sequencing of the in-situ and invasive AC was conducted subsequently matched the adjacent normal tissue of this patient. On sequencing the invasive tumor sample was found to have 27 exclusive somatic mutations as compared with in-situ and adjacent normal tissues. Our case exemplifies the need for deep sequencing and the discovery of new potential driver mutations.

Keywords: Multiple lung adenocarcinomas, whole exome sequencing, somatic mutation

Introduction

Currently, increasing clinically-confirmed cases certify the unique benefits of genotype-directed personalized therapy in improving efficiency to treat non-small cell lung carcinoma (NSCLC) when compared with traditional chemo-therapy [1, 2]. Nevertheless, lung cancer, of which NSCLC accounts for nearly 85%, remains the leading cause of cancer deaths globally nowadays [3, 4]. The two major forms (about 70%) of NSCLC are AC and squamous-cell carcinoma (SCC) [5]. For one thing, the majority of lung cancer patients reaches an advanced stage at the time of the diagnosis, thereby have an unfavorable prognosis [5, 6]. For another, targetable small molecule inhibitors aiming at NSCLC are still desperately limited [7]. Furthermore, only 40% to 50% driver oncogenes of SCC have been examined, while approximately 60% driver mutations of AC have already been confirmed [7]. For these reasons, to discover potential lung cancer drug targets seems to be more pressing to some extent. To this end, cutting-edge methods such as the next generation sequencing (NGS) or traditional methods including pyro-sequencing [8], Sanger sequencing [9] and PCR-based assays [10] are widely needed. Among them, the NGS offers unbiased analysis of mutations and complex structural changes including translocation and gene copy number changes, thus providing more complete and accurate information about a patient's cancer, which may guide more appropriate clinical decision-making [11].

Herein, we present a case of synchronous primary AC of a Chinese woman without lymph nodes metastases. With the traditional pathologic examination, the lung neoplasms were diagnosed as AC. EGFR mutation test, fluorescent in situ hybridization (FISH) analysis for ALK gene and the whole exome sequencing were conducted so that a more proper treatment can be given. Surprisingly, that in the invasive carcinoma node exclusively which could be therapeutic targets in the future.

Case presentation

A 62-year-old Chinese woman, who had never been a smoker, was referred to our hospital for further diagnosis and management due to

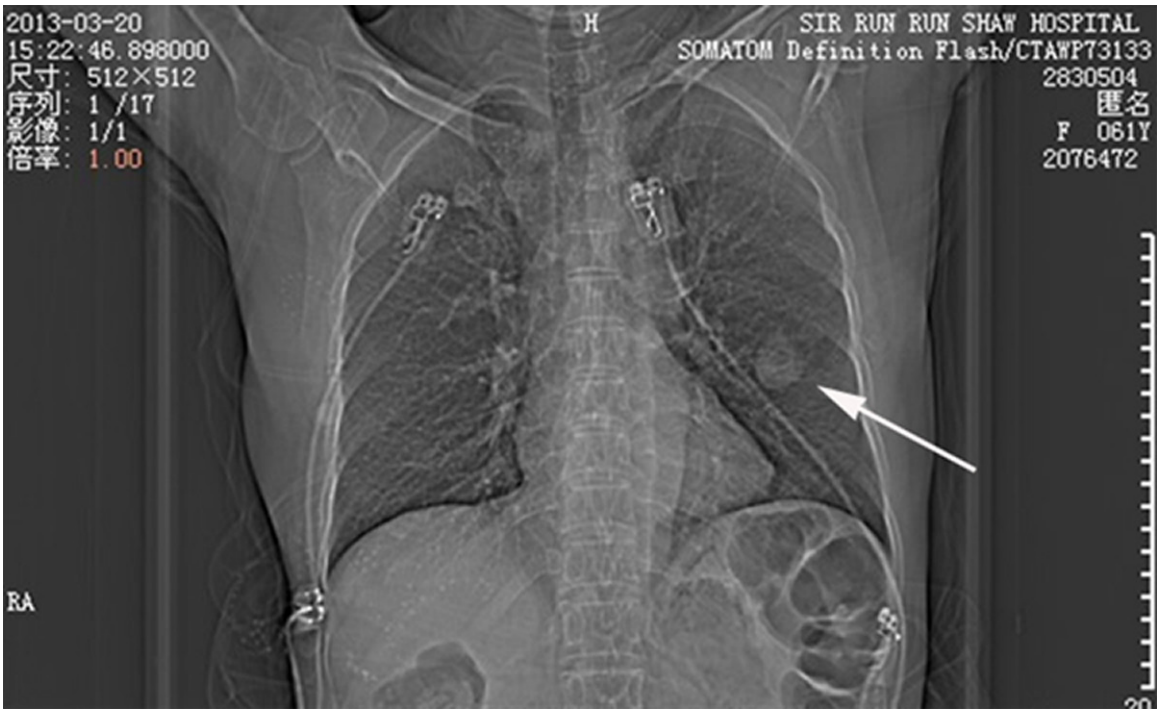

Figure 1. The coronal CT image. The lesion of malignancy in the left lower lobe was shown by the arrow.

abnormal chest ECT finding at another hospital. The chest CT was conducted because of her chest discomfort for more than half a year, getting worse when doing exercise, accompanied by numbness sometimes. A lesion highly skeptical of malignancy was found in the left lower lobe (**Figure 1**), and so was an ill-defined weak ground-glass opacity in the left upper lung (data not shown). Additionally, the results of the pulmonary function were approximately within the normal range. Laboratory findings including peripheral blood were within normal scope and abdominal ultrasonography result demonstrated no abdominal metastatic lesions. Accordingly, the left lower lobectomy was considered to be operated. Intraoperative detection, however, showed a solid nodule in posterior segment of the left upper lobe, measuring around 1 cm in diameter and a 3.0-cm-sized tumor in the left lower lobe dorsal segment, as well as enlargements of several interlobe lymph nodes and parabronchial mediastinal lymph nodes in the lower lobe. Therefore, the left upper lung wedge resection and the left lower lobectomy were performed, so was the lymph-node dissection. While the left upper tumor was confirmed to be in-situ AC (**Figure 2A**) with alveolar epithelial dysplasia but not significant invasion on the histological examination, the left lower lobe tumor was pathologically well-differentiated AC (**Figure 2A**). Meanwhile, the examined 21 enlarged lymph nodes turned out to be negative.

These tumors were fixed in formalin and embedded in paraffin, then stained with hematoxylin and eosin (HE). Intriguingly, the adjacent normal tissue was available. For EGFR gene mutational analysis, genomic DNA was extracted from these tissue sections, and then sequenced for exon18, 19, 20 and 21 as previously described [12]. EGFR gene exon19 deletion (2235_2249del15) was detected in the invasive AC (**Figure 2B** and **2C**). Although EGFR mutation and other confirmed mutations like KRAS or ALK are mutually exclusive [13], there are possibilities that multiple mutations are found within a single gene or across multiple genes according to the NCCN clinical practice guidelines in Oncology on mutation test in NSCLC [14]. Thereby FISH for ALK rearrangement was performed as previously described [15]. It turns out that the FISH test for ALK was considered negative (**Figure 2D**). To find out invasion related somatic mutation, we did the whole exome sequencing of DNA extracted

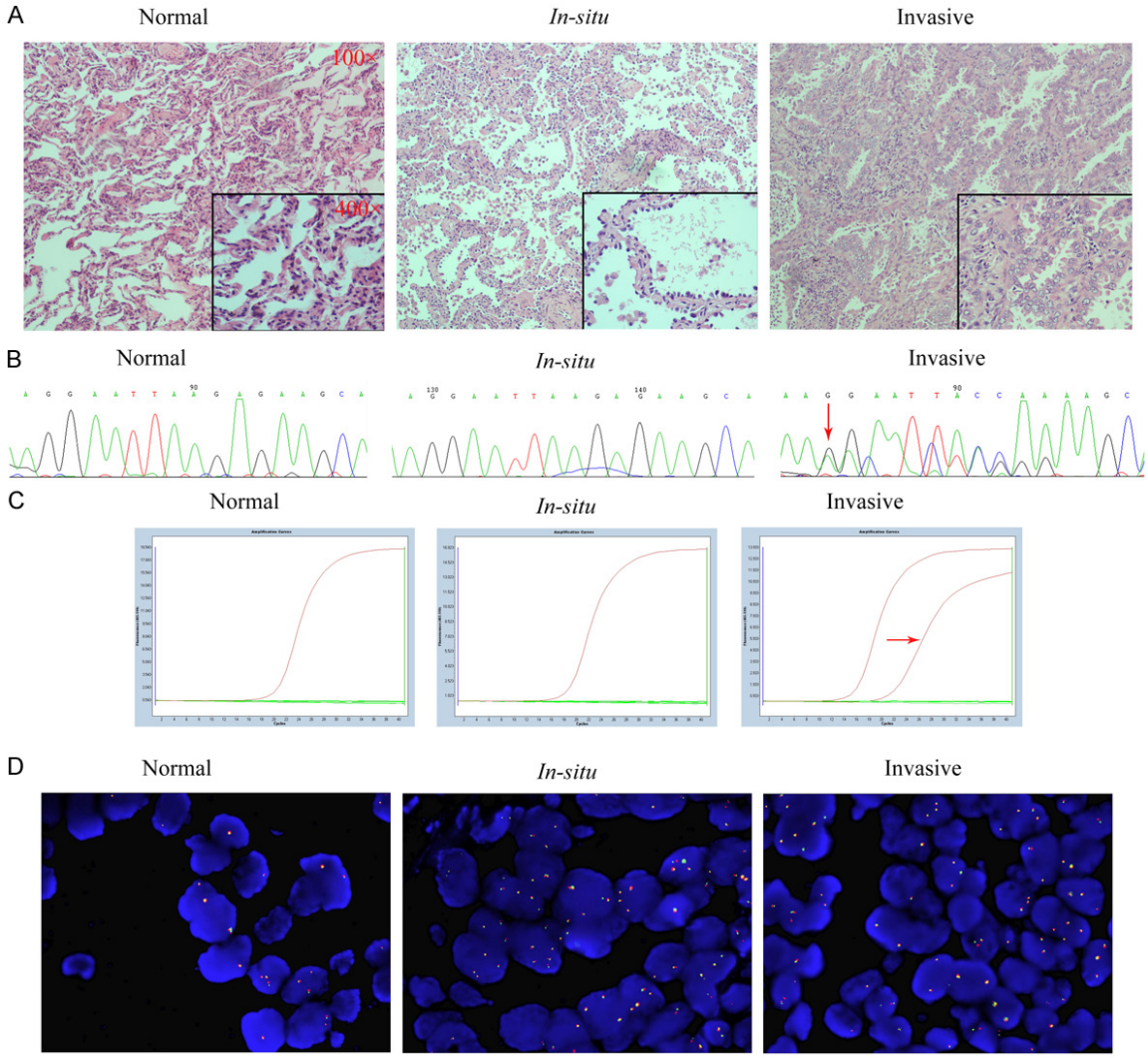

Figure 2. A. H&E staining of normal tissue, in-situ and invasive carcinoma tissues from the patient; B. Sequencing electropherograms of EGFR gene exon19; C. Real-time PCR analysis of the three samples; D. ALK FISH analysis of the three samples. Mutation site or mutation amplification curve were pointed out by red arrows.

from these tissue sections. Somatic mutation analysis indicated that normal, in-situ and invasive tissue samples showed different signal intensity of 214 somatic mutations (**Figure 3**). Strikingly, twenty-seven mutations (**Table 1**) were pointed out only in the invasive tumor samples, which strongly suggested that these sites may be potential mutational sites and may play pivotal roles in tumor invasion.

Discussion

Lung cancer, approximately 85% of which is composed of NSCLC, represents the leading cause of cancer-related death worldwide recently [3, 4]. The personalized treatment of NSCLC, which is usually diagnosed at an advanced clinical stage [5], proved to be superior to traditional chemo-therapy [1, 2]. It is plausible that a deeper investigation is urgently required to uncover other gene mutations. Among lung cancer, multiple AC is relatively infrequent [16]. Most researches concerning lung AC studied the potential mutation sites using the in-situ neoplasm or the invasive one along with the matched normal tissue from different patients [17-19]. In our case, one patient's tissues of the normal, in-situ carcinoma and the invasive carcinoma were all sequenced, which may contribute to discovery of new driver mutations that might have a vital role in cancer formation, invasion or metastasis. These specimens were collected from one patient, which can attenuate the influence of inter-patient genome heterogeneity. Indeed, 214 somatic mutations

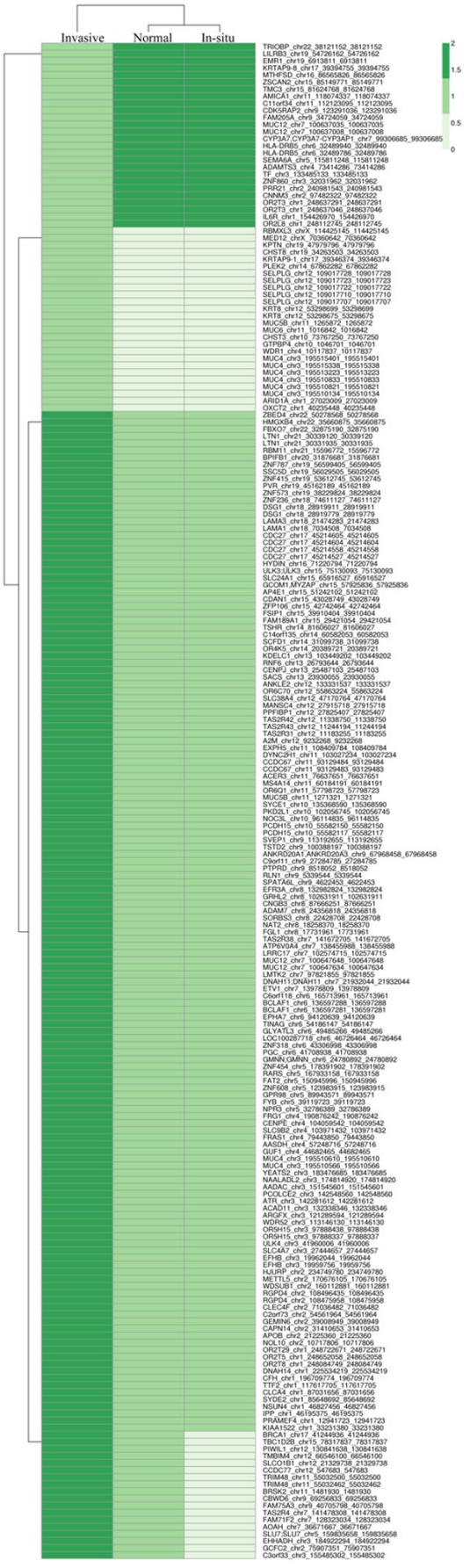

Figure 3. Heat-map of somatic mutation analysis. Normal, in-situ and invasive carcinoma tissues showed different signal frequency of various somatic mutations.

were found to have different signal intensities among the three different samples, furthermore, 27 somatic mutation sites were discovered in the invasive carcinoma sample exclusively. Very few short fragment deletions or insertions were found in our study, simply because whole exome sequencing has less efficiency to identify deletions or insertions.

As is described in the **Table 1**, all the biological functions of these mutated genes were listed. For instance: FBXL20 participates in protein ubiquitination, while ARID1A is involved in altering the chromatin structure. Zhu et al. proposed that FBXL20 promoted the carcinogenesis of human colorectal adenocarcinoma [20]. And over-expression of FBXL20 gene was reported to lead to an increased invasive ability of colorectal adenocarcinoma cells [21]. In contrast, in our research, the frameshift deletion G7fs of FBXL20 was discovered alone in the invasive lung AC tissue. ARID1A, whose mutations were identified in several tumors including lung carcinomas [22], was also validated to be mutated in lung AC [18]. Conversely, the ARIDIA nonsynonymous mutation A39P in our sequencing differs from those found in other studies [18]. Functional research concerning mutations listed in **Table 1** to tumor carcinogenesis and invasion deserves to be performed in the future. To our best knowledge, this is the first study, sequencing tissues of the normal, in-situ carcinoma along with the invasive carcinoma from one patient. Yet it should be noted that we have not validated these somatic mutated sites for lacking of these valuable specimens. According to the NCCN clinical practice guideline in oncology on mutation test in NSCLC, multiple mutations may exist in a single gene or across multiple genes [14]. Hence, it is of great significance for researchers to find and confirm more driver mutation sites which may serve as therapeutic targets to improve the treating efficiency of NSCLC.

In summary, more attention should be paid to find specimens like these ones and confirm the potential driver mutations we found in the future.

Table 1. Somatic mutations in the invasive carcinoma tissue

Genes	Mutations	Gene functions
ARID1A	exon1:c.G115C:p.A39P	Regulate transcription of certain genes by altering the chromatin structure around those genes
OXCT2	exon1:c.G1480A:p.D494N	Catalyzes the reversible transfer of CoA from succinyl-CoA to acetoacetate
MUC4	exon2:c.A8317G:p.N2773D	Protection of the epithelial cells and have been implicated in epithelial renewal and differentiation
MUC4	exon2:c.T7630C:p.S2544P	Same as above
MUC4	exon2:c.C7618G:p.R2540G	Same as above
MUC4	exon2:c.G5228C:p.S1743T	Same as above
MUC4	exon2:c.T3113C:p.V1038A	Same as above
MUC4	exon2:c.T3050C:p.V1017A	Same as above
WDR1	exon2:c.C38G:p.P13R	Help induce the disassembly of actin filaments.
GTPBP4	exon7:c.C739G:p.L247V	GTP-binding proteins
CHST3	exon3:c.A461G:p.Q154R	Catalyzes the sulfation of chondroitin
MUC6	exon31:c.A5959C:p.T1987P	Epithelial cytoprotection
MUC5B	exon31:c.G7762A:p.G2588R	Mucus secretions
KRT8	exon2:c.T91G:p.S31A, KRT8	Maintaining cellular structural integrity and also functions in signal transduction and cellular differentiation
KRT8	exon2:c.C67T:p.R23C	Same as above
SELPLG	exon2:c.C377T:p.P126L	A high affinity counter-receptor for the cell adhesion molecules
SELPLG	exon2:c.A374C:p.Q125P	Same as above
SELPLG	exon2:c.T362C:p.I121T	Same as above
SELPLG	exon2:c.A361G:p.I121V	Same as above
SELPLG	exon2:c.T356C:p.M119T	Same as above
PLEK2	exon3:c.A226G:p.T76A	Cytoskeleton
KRTAP9-1	exon1:c.C236T:p.S79F	Keratin-associated protein 9
FBXL20	exon1:c.20delG:p.G7fs	Protein-ubiquitin ligases
CHST8	exon4:c.C810A:p.D270E	Sulfotransferase 2
KPTN	exon11:c.T1175G:p.I392S	Actin binding protein
MED12	exon42:c.C6202T:p.Q2068X	Initiation of transcription
RBMXL3	exon1:c.G1141C:p.D381H	RNA binding motif protein

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Disclosure of conflict of interest

None.

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