Original Article

Genomic alteration profiles of lung cancer and their relationship to clinical features and prognosis value using individualized genetic testing

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Background: This study aimed to use a panel targeting 197 genes and 38 fusions to observe the features of gene variations in lung cancer patients, as well as their prognostic values.

Methods: Patients admitted to our hospital between 2016 and 2017 were enrolled. All patients received OseqTM-Drug genetic testing using peripheral venous blood, followed by 1–2 years of observation.

Results: For all included patients, 32 genes were observed with mutations. EGFR exhibited the highest mutation rate (46.5%), followed by TP53. The majority of patients carried only one mutant gene. Interestingly, 18 (41.8%) patients showed no mutations, and some cases carried mutations in six genes simultaneously. There was no statistical relationship between mutations and demographic influence. Pathological subtypes were associated with mutations including *FLI1*, *IGF1R*, and *NOTCH1*. A significant correlation was observed between mutant genes and stage at diagnosis, however this requires further confirmation as there was only one case in these mutations: *AKT2*, *AR*, *STK11*, *VEGFA*, *HDAC6*, and *ASPSCR*. For the 33 patients with lymph node metastases at the time of diagnosis, no correlation with any gene mutant was found. Finally, no associations between the survival or prognosis indices (1-year survival, 1-year progression, progression free survival (PFS), and overall survival (OS)) were observed with gene mutations.

Conclusions: Together, individualized genetic testing is a feasible and minimally invasive approach in cancer genetic analysis. However, gene mutation detection has a limited efficacy in the prediction of prognosis.

Keywords: Lung cancer; genomic DNA; mutation; individualized genetic testing; sequencing

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Introduction

Genetic testing using the next-generation sequencing (NGS) analysis is becoming increasingly recommended for cancer diagnosis and treatment decision-making (1,2). Traditionally, the NGS technique was applied to tumor tissues to examine gene alterations related to specific cancers (3,4). Currently, an increasing number of medical centers have recommended the liquid biopsy technique to admitted patients, owing to its advantages of minimal invasion and ability to probe the genomic profile of tumors in real time (5-7). In China, the choice between different commercial genetic testing products to conduct sequencing based on blood samples is available; for many tumor departments, clinical data accompanied by genetic testing results have been accumulated in recent years. A timely analysis of the data features in the real world would greatly push forward

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Table 1 All detected genes in the testing panel

ABL1, ACVRL1, AKT1, AKT2, AKT3, ALK, APC, AR, ARAF, ASPSCR1, ATF1, ATM, ATP11B, ATR, AURKA, BAP1, BCL2, BCL2L1, BCR, BIRC2, BIRC3, BRAF, BRCA1, BRCA2, BRD4, BTK, C11orf30, CBL, CBR3, CCDC6, CCND1, CCND2, CCNE1, CD44, CD74, CDH1, CDK4, CDK6, CDKN2A, CDKN2B, CHEK2, CREB1, CRTC1, CSF1R, CSNK2A1, CTLA4, CTNNB1, DDR1, DDR2, DNMT3A, EGFR, EML4, EPHA2, EPHA3, ERBB2, ERBB3, ERBB4, ERG, ESR1, ESR2, ETV6, EWSR1, EZH2, EZR, FBXW7, FCGR2A, FCGR2B, FCGR3A, FGD4, FGFR1, FGFR2, FGFR3, FGFR4, FLCN, FLI1, FLT1, FLT3, FLT4, FOXL2, FUS, GAB2, GATA3, GNA11, GNAQ, GNAS, HDAC1, HDAC2, HDAC3, HDAC4, HDAC6, HDAC8, HGF, HNF1A, HRAS, IDH1, IDH2, IGF1R, IL6, IRS2, JAK1, JAK2, JAK3, JAZF1, KDR, KIAA1549, KIF5B, KIT, KRAS, MAML2, MAP2K1, MAPK1, MAX, MCL1, MDM2, MDM4, MED12, MET, MLH1, MLH3, MPL, MS4A1, MSH2, MSH3, MSH6, MTOR, MYB, MYC, MYCN, MYD88, NCOA4, NDRG1, NF1, NF2, NFIB, NOTCH1, NOTCH2, NOTCH3, NOTCH4, NR4A3, NRAS, NTRK3, NUTM1, PARP1, PARP2, PAX5, PAX8, PBX1, PD-1, PDGFRA, PDGFRB, PD-L1, PIK3CA, PIK3CB, PIK3R1, PLAG1, PMS1, POU5F1, PPARG, PPP2R1A, PRCC, PRKAA1, PSMB5, PTCH1, PTEN, PTPN11, RAC1, RAF1, RANKL, RB1, RET, RHEB, RHOA, RICTOR, ROS1, RPS6KB1, SF3B1, SLC34A2, SLC45A3, SMAD2, SMAD4, SMARCA4, SMARCB1, SMO, SND1, SOX2, SPOP, SRC, STAT3, STK11, SUZ12, TAF15, TCF3, TERT, TET2, TFE3, TMPRSS2, TP53, TPM3, TRIM33, TSC1, TSC2, U2AF1, VEGFA, VHL, WT1, XPO1, ZNF217

the progress of understanding the onset and development of cancer.

NGS has been used to clarify the gene mutations differences of ctDNA in peripheral blood between early-stage lung cancer and benign nodules. RNF213 mutations is identified to be the biomarker of early-stage lung cancer (8). Targeted deep sequencing is also performed to compare the proportion of positive DNA mutations (40 mutations identified) in the four samples (primary tumors, pulmonary venous blood, peripheral blood, and rib bone marrow fluid) in 10 lung patients (9). However, the samples size is small in previous studies. The pulmonary venous blood is the most convenient way to detect the gene mutation. As the NGS technological development, the sensitivity to detect mutations in peripheral venous blood is increasing. Large sample research is needed to verify the efficiency of NGS on gene mutation in peripheral venous blood.

Among all malignancies, lung cancer is the leading cause of cancer-related death in China and worldwide. Focusing on lung cancer, some recognized genes (like *TP53*, *KRAS* and *EGFR*, etc.) have been widely surveyed for their roles in development and relapse. For example, many lung cancer patients carry mutated EGFR and are generally recommended targeted therapy. Despite the widely studied roles of genes such as EGFR, it remains challenging but rewarding to probe a panel of other gene alterations . To date, sufficient data have been accumulated, including the sequencing results and clinical characteristics of lung cancer patients. However, limited studies have analyzed their relationships and the prognostic role of the genetic testing profiles in depth. Therefore, we conducted this study and aimed to use a panel targeting 197 genes and 38 fusions to observe the features of gene variations in lung cancer patients, as well as their prognostic values. Compared with previous study the sample size was large and the panel content was larger. This study will provide more information on gene mutation in cancer genetic analysis. We present the following article in accordance with the MDAR reporting checklist (available at https://dx.doi.org/10.21037/jtd-21-1031).

Methods

Patients

Patients admitted in our hospital between 2016 and 2017 were eligible for inclusion in this study. The inclusion criteria were as follows: (I) primary lung cancer patients; and (II) those who had received Oseq^{TM}-Drug (Beijing Genomics institution, Beijing) genetic testing. Patients were excluded based on the following criteria: (I) those unwilling to sign the informed consent; and (II) incompatibility in the follow-up data collection. Finally, 33 patients were enrolled. This cohort comprised 25 males and eight females, with a mean age 58.9 years old (range, 43–82). Subjects received the mutation examination of a panel including 197 genes and 38 gene fusions, as shown in *Tables 1* and *2*. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The present study was approved by the ethics committee of the Fourth Hospital of Hebei Medical University (No.: 2021KY255). The informed consent was taken from all the patients.

Sample collection and genomic DNA extraction

The samples were collected and detected as soon as possible. Briefly, for each patient, a total of 5 mL peripheral venous blood was collected in Ethylene Diamine Tetraacetic

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Table 2 All detected gene fusions in the testing panel

BCR-ABL1, CCDC6-RET, CD74-ROS1, EML4-ALK, EWSR1-ATF1, EWSR1-CREB1, EZR-ROS1, KIF5B-ALK, KIF5B-RET, RET-NCOA4, SLC34A2-ROS1, TMPRSS2-ERG, TPM3-ALK, TPM3-ROS1, TRIM33-RET, ASPSCR1-TFE3, BRD4-NUTM1, CRTC1-MAML2, CTNNB1-PLAG1, ETV6-NTRK3, EWSR1-ERG, EWSR1-FLI1, EWSR1-NR4A3, EWSR1-PBX1, EWSR1-POU5F1, FGFR1-PLAG1, FUS-ERG, JAZF1-SUZ12, KIAA1549-BRAF, MYB-NFIB, NDRG1-ERG, PAX8-PPARG, PRCC-TFE3, SLC45A3-BRAF, SLC45A3-ERG, SND1-BRAF, TAF15-NR4A3, TCF3-PBX1

Acid (EDTA) anticoagulation tubes and stored at 4 ℃ prior to treatment. The circulating tumor DNAs (ctDNAs) and genomic DNAs were extracted. Briefly, 2 mL of blood was centrifuged at 2,000 g for 10 min, red blood cells and buffy coat were discarded, and the plasma was centrifuged at 16,000 g for 10 min at 4 ℃. A volume of 500 μL purified plasma was treated with the TIANamp Blood DNA Kit (Tiangen Biotech, China).

Genotyping

The pre-hybridization DNA sample was treated through terminal repair, linker ligation, and pre-polymerase chain reaction (PCR) amplification. Next, the DNA fragment containing the target region was specifically captured by the biotin-labeled DNA probe, followed by the post-PCR process. After hybridization, the sequencing reaction was carried out using the OseqTM-drug Individualized Gene Testing Kit (Beijing Genomics institution, Beijing). The sequencing information of each target region was acquired and exported. After compared to the normal human genome sequence, any variation will be documented. For each subject, an individualized report was generated, which included the mutant gene name, nucleotide change, heterozygosity, inheritance pattern, and mutation types.

Statistical analysis

Data were mainly expressed as a frequency, and Chi-square analysis of the frequency distribution was used to determine the correlation between two nominal variables. $P<0.05$ was considered statistically significant.

Results

Clinical features

In total, 43 subjects were initially enrolled, including 26 males (mean age 59.1 years old, 43–75) and 17 females (mean age 58.3 years old, 34–82). Three patients who had not clearly received a subtype determination through section pathological examination were excluded. Among the remaining 40 participants, there were 35 cases of adenocarcinoma (ADC) and five cases of proven squamous cell carcinoma (SCC). No other sub-types were observed in this study. At the time of diagnosis, there were four cases in stage I, one case at stage II, 10 cases at stage III, and 28 cases at stage IV. Among all 43 subjects, 33 showed lymph node metastases at the time of diagnosis.

Mutation features

Within all the targets in the panel, 32 genes were observed with mutations, which were ranked according to their mutation frequency (*Table 3*). As expected, the EGFR gene exhibited the highest mutation rate, with 46.5% subjects (20 cases) carrying mutations of this gene. Also, the well-known oncogene, TP53, ranked second in mutation frequency. Other common mutations included NF1, FBXW7, HGF, etc. We further counted the frequencies of individuals with different mutation gene numbers. Patients carrying only one mutant gene exhibited the highest proportion, with 13 patients (28.9%) carrying mutants in one gene. There were four patients (9.3%) with mutants in two genes, three patients (7.0%) with mutants in three genes, one (2.3%) with mutants in four genes, and interestingly, three patients (7.0%) with mutants in five genes. For some of the zero-mutation subjects, we further checked this conclusion using the biopsy samples from the tumor tissue and ensured no detectable mutations. This result implied that there are still a large proportion of lung cancer patients without recognized mutations in our panel.

Correlations between mutations and cancer states

Next, we analyzed the association between gene mutation and disease states, such as the stage, subtype, and metastasis at the time of diagnosis. There were no statistical relationships between mutations and tumor stage, sex, age, etc. However, pathological subtypes were associated with several gene mutations (*Table 4*): *FLI1*, *IGF1R*, and

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Table 3 Case numbers of 32 detected mutant genes

Gene	Mutation cases
EGFR	20
TP53	6
MSH6	3
HER2	2
ZNF217	2
BRCA1	2
NF1	1
FBXW7	1
AKT2	1
BRAF	1
HGF	1
EPHA2	1
BIRC2	1
AR	1
KIF5B-RET	1
PARP1	1
AKT3	1
ATM	1
ERBB4	1
MSH2	1
MAP2K1	1
MED12	1
SOX2	1
NOTCH1	1
FLI1	1
IGF1R	1
NOTCH3	1
STK11	1
VEGFA	1
HDAC6	1
ASPSCR1	1
AKT1	1

NOTCH1. A significant correlation between mutant genes and stage at diagnosis was observed (*Table 5*), however this requires further confirmation, as there was only one case in these mutations: *AKT2*, *AR*, *STK11*, *VEGFA*, *HDAC6*, and *ASPSCR*. Moreover, for the 33 patients with lymph node metastases at the time of diagnosis, no correlation between metastasis and gene polymorphism was found.

Correlations between gene mutations and prognosis

Furthermore, the prognostic value of mutation states was probed after the follow-up data collection. Relapse and later metastasis were surveyed regarding their relationship with gene mutations. There was no association between 1-year progression and gene mutations (*Table 6*). Similarly, there were no differences in the 1-year survival among patients with different gene mutations. Next, we compared the PFS and OS using the Kaplan-Meier (KM) method. To acquire a reliable conclusion, those gene mutations with only one positive case were not included. Namely, the following genes were analyzed: *EGFR*, *TP53*, *MSH6*, *HER2*, *ZNF217*, and *BRCA1*. None of the above gene mutations were found to be correlated with PFS. Moreover, OS was assessed, and none of the above six genes exhibited a relationship with OS. Taken together, gene mutation detection has a limited efficacy in the prediction of prognosis.

Discussion

In the present study, we applied a non-invasive method to detect peripheral blood DNA, and performed individualized genetic testing to survey the genomic alterations in lung cancer patients. NGS is a potential method in cancer research. NGS has many NGS has been shown to have a powerful effect on many aspects of lung cancer. NGS could detect the gene mutation (10), guide the application of sensitive drug (11), promote diagnosis (12) and prognosis (13) in lung cancer research. The mutant genes could be detected and they are associated with the tumorigenesis of lung cancer. In early stage of tumorigenesis, simultaneous mutation of EGFR and ALK could be induced through different tumor evolution (14). Further, many genes are mutant and differently expressed in lung cancer, the histone 3 lysine-27 demethylase KDM6A is associated with tumorigenesis and prognosis (15). COX1, COX2, COX3, ND1, ND2, ND4L, and ATP6 are abnormal increase in the plasma of non-small cell lung cancer (16). Thus, using NGS to clarify the mutant genes in peripheral blood is of great importance in lung cancer research.

In our high-throughput panel, we observed 32 genes with mutations. Also, approximately 18% of patients did

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Table 4 Correlation between mutant genes and pathological subtypes

Genes	Pathological subtypes			Chi-square	P value
	ADC	SCC	Undefined		
NF1				7.781	0.020
WT	35	4	3		
MT	0	1	0		
AR				13.651	0.001
WT	35	5	2		
MT	0	0	1		
KIF5B-RET				13.651	0.001
WT	35	5	2		
MT	0	0	1		
AKT3				13.651	0.001
WT	35	5	2		
MT	0	0	1		
NOTCH1				7.781	0.020
WT	35	4	3		
MT	0	1	0		
AKT1				13.651	0.001
WT	35	5	2		
MT	0	0	1		

ADC, adenocarcinoma; SCC, squamous cell carcinoma; WT, wild type; MT, mutant.

not exhibit any mutation in either blood or tumor tissue samples, which may provide a different understanding of lung cancer onset. Moreover, we observed clear associations between gene mutations and cancer states at diagnosis.

Consistent with the widely established conclusion, EGFR mutation plays a prominent driving role in lung cancer onset (17,18). In our study, 46.5% of participants were diagnosed with EGFR mutation. These patients can largely benefit from the fluid biopsy followed by genetic testing, as targeted therapy can be selected for them using EGFR tyrosine kinase inhibitors (TKIs). For East Asians, EGFR exhibits the highest mutation frequency, and this ratio is lower in Americans/Europeans (19,20). TP53 ranked second in mutation frequency behind EGFR. This finding was seldomly noticed, as BRAF or KRAS, but not TP53, were generally among the most frequently observed mutations in lung cancer following EGFR. Our result further emphasized the tumor-suppressor role of TP53 in lung cancer development.

Interestingly, approximately 18% patients did not carry any known mutation. This may be due to the limited throughput of our testing panel, limited sensibility of the testing protocol, tumor heterogeneity, or some undiscovered mechanisms of lung cancer onset. We will pursue further research in the future, especially focusing on those cases without detectable mutations.

Based on our correlation analysis, some important genes were found to potentially influence the pathological subtypes. For example, SCC patients had higher mutation frequencies in *FLI1*, *IGF1R*, and *NOTCH1*, and a higher risk of lymph node metastasis. It is agreed that SCC exhibits increased metastasis and drug resistance, with a higher expression of markers being associated with lymph node metastasis (21-23). However, this is the first report linking *FLI1*, *IGF1R*, and *NOTCH1* mutations with the lung SCC subtype. A previous report identified *NOTCH1* or *NOTCH2*

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Table 5 Correlation between mutant genes and stage at diagnosis

Genes	I	II	III	IV	Chi-square	P value
AKT2					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		
AR					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		
STK11					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		
VEGFA					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		
HDAC6					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		
ASPSCR1					9.982	0.019
WT	3	1	10	28		
MT	1	0	0	0		

WT, wild type; MT, mutant.

mutations in 75% of cutaneous SCCs but in a lesser fraction of lung SCCs (24). Our findings provide a new insight into SCC development or transition.

However, the same gene may play different or even contrary roles in different lung cancer subtypes. For instance, in lung adenocarcinoma, Numb protein impairs tumor growth and inhibits the Notch pathway and epithelial-mesenchymal transition, whereas in lung squamous cell carcinoma, it may promote proliferation (25). In addition, we noticed that some mutant genes significantly influenced the metastasis/recurrence outcomes, especially ATM. Combined with metastasis at the time of diagnosis and in the post-treatment follow-up period, ATM mutation is the most frequent factor in all kinds of metastases. Published data supports that ATM sequence variants and expression could be used as a predictor for radiotherapy responses and chemotherapy resistance in breast cancer patients (26,27). Moreover, ATM mutation is an important driving factor of tumorigenesis (28-30). Following ATM, STK11 mutation was another important factor that repeatedly appeared in metastases. It was mentioned that inactivation of STK11 was closely related to tumor occurrence, dominantly in non-small cell lung cancer (31,32). Some scholars also claimed that STK11 mutation may also play a role in identifying thyroid carcinoma (33). Therefore, it is valuable to probe the detailed role of ATM and STK11 in suppressing tumor development and metastasis.

There are some limitations in the present work that should be noted. Firstly, the sample size was small, and many significances were derived from the crucial one or two individuals with multiple mutations. Also, due to the small sample size, our conclusions cannot be majorly applied in ADC and SCCs, and there is an obvious void with regards to small cell lung cancer. Furthermore, the follow-up period was so short that all the participants were still alive at the time of data analysis. Therefore, all of the results regarding the association between gene mutations and prognosis were negative. Moreover, the targets of the panel needed to be extended and the sensitivity requires enhancement, especially considering that 18% patients were negative in

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Table 6 No correlation between mutant genes and 1-year progression

Genes	Chi-square	P value
EGFR	0.635	0.426
NF1	0.342	0.559
FBXW7	0.342	0.559
AKT2	0.342	0.559
BRAF	3.077	0.079
TP53	0.261	0.609
HGF	3.077	0.079
MSH6	0.120	0.729
EPHA2	3.077	0.079
BIRC2	3.077	0.079
AR	3.077	0.079
KIF5B-RET	3.077	0.079
PARP1	0.342	0.559
AKT3	3.077	0.079
ATM	0.342	0.559
HER2	0.702	0.402
ZNF217	0.702	0.402
ERBB4	0.342	0.559
MSH2	0.342	0.559
MAP2K1	0.342	0.559
MED12	0.342	0.559
SOX2	0.342	0.559
NOTCH1	0.342	0.559
FL1	0.342	0.559
IGF1R	0.342	0.559
NOTCH3	0.342	0.559
BRCA1	0.702	0.402
STK11	0.342	0.559
VEGFA	0.342	0.559
HDAC6	0.342	0.559
ASPSCR1	0.342	0.559
AKT1	3.077	0.079

the mutation detection. So far, it is too early to ascertain whether the reason for this phenomenon lies in the testing means or the patients themselves. Lastly, this correlation study could not reveal the detailed mechanisms of some findings, especially those inconsistent with known reports (e.g., EGFR mutation was negatively correlated with lymph node metastases).

In summary, we evaluated the usefulness of tumor DNA sequencing from blood samples of lung cancer patients, which is a feasible and minimally invasive approach in cancer genetic analysis. EGFR and TP53 are the first two mutant genes; NF1, AR, KIF5B-RET, AKT1, AKT3 and NOTCH1 were associated with the pathological subtypes. AKT2, AR, STK11, VEGFA, HDAC6 and ASPSCR1 were associated with the stage at diagnosis. Although the usefulness of gene mutations in prognosis prediction is limited in this study, the mutant genes detected by NGS could also provide essential guide information for the diagnosis, prognosis and treatment drug selection lung cancer.

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All procedures performed in this study involving human participants were in accordance with the Declaration of Helsinki (as revised in 2013). The present study was approved by the ethics committee of the Fourth Hospital of Hebei Medical University (No.: 2021KY255).

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