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Genomic profiling of non-small cell lung cancer: A pilot study from South India

Linu A. Jacob[1], K. C. Lakshmaiah[1], K. Govindbabu[1], D. Lokanatha[1], Ankit Agarwal[1], Abhishek Anand[1], Swapnil R. Jaiswal[2], Venkat Addala[2], Shaleen Multani[3]

[1]Department of Medical Oncology, Kidwai Memorial Institute of Oncology, KMIO Campus, Bengaluru, Karnataka, [2]Positive Bioscience Ltd., [3]Department of Biological Science, Sunandan Divatia School of Science, NMIMS University, Mumbai, Maharashtra, India

Correspondence to: Sahleen Multani, E-mail: shaleen.multani@gmail.com

ABSTRACT

Introduction: Lung cancer accounts for the highest mortality globally, and there is a need to find new strategies and novel treatment options. Lung cancer is genetically heterogeneous across various ethnic populations, and therefore identifying the mutational landscape in Indian patients is important. **Methods:** The aim of the pilot study is to identify the prevalence and temporal sequence of molecular lesions in lung cancer patients from a regional cancer institute in South India using the circulating tumor DNA technique. Genotyping was performed on 26 newly diagnosed metastatic lung cancer patients using Illumina Omni Express Exome. Chip data were inspected using Genome Studio and subsequently exported for use with PLINK and further annotation was performed. **Results:** Seven genes including TP53 (61.5%), MUC16 (57.6%), KRAS (38.4%), STK11 (19.2%), Epidermal growth factor receptor (15.3%), ataxia telangiectasia mutated (15.3%), and nuclear factor-κβ (7.6%) demonstrated high incidence of mutations in patients. The other genes identified were observed in <5% of patients. Several types of mutations including missense, silent, nonsense, and frameshift mutations were observed in these genes. **Conclusion:** Integration of mutational profiling to identify gene mutations is required to facilitate personalized lung cancer therapy.

Key words: Genomic profiling, Circulating tumor DNA, India, Lung cancer, mutation, Non-small cell lung cancer

Introduction

Lung cancer is the most common cause of death worldwide accounting for 19.4% of mortality. In India, it is the most common cancer among males and fourth most common cancer in the population.[1] Lung cancer is divided into two histological subtypes - small cell lung cancer (SCLC) and non-SCLC (NSCLC). The majority of lung cancers belong to NSCLC type, which are further subdivided into squamous cell carcinoma, large cell carcinoma, and adenocarcinoma.[2] Despite the advances in treatment option, the overall 5-year survival is only 15% and therefore there is a need to find new strategies and novel treatments. Several genes are involved in tumorigenesis of lung cancer and help in the molecular subtyping which would further enhance treatment.[2]

Identification of the key dysregulated genes and pathways is necessary to develop preventive, diagnostic and therapeutic targets. The genetic heterogeneity of lung cancer among various ethnic populations has brought to the stage the need for regional studies on molecular mechanisms underlying lung cancer phenotypes. Even though there are several published studies on molecular profiling of lung cancer from the Western world and Japan, there are very few from the Indian subcontinent. In the French population, the molecular profiling of NSCLC is recommended for routine care.[3] A recent study to identify common driver mutations in the Japanese population was performed and suggested that molecular profiling is essential to expand the range of molecular targeted therapy in NSCLC.[4] Adequacy of tumor tissue for molecular profiling is an important issue and even more relevant in lung cancer where the yield is limited by small core biopsies. Circulating tumor DNA (ctDNA) is a useful tool in these situations and can be used for mutation testing and therapeutic monitoring.[5] The other major advantages of ctDNA are that it can detect mutations occurring in all the subclones of cancer cells. Since cancer is a heterogeneous disease, tissue acquired from one site does not provide a holistic picture of cancer in advanced stages.[6] The aim of the pilot study was to study the prevalence and temporal sequence of molecular lesions in lung cancer patients from a regional cancer institute in South India using the circulating cell-free DNA (cfDNA) technique.

Methods

Sample selection and DNA extraction

Newly diagnosed patients with metastatic NSCLC at Kidwai Cancer Institute from November 1, 2015, to December 30, 2015, were considered for the study. Blood samples (10 ml) were collected into Becton-Dickinson vacutainer serum tubes, double

centrifuged at 2000 ×*g* for 10 min at room temperature, and the plasma stored at −80°C within 4 h of collection. Ethics approval was obtained from Kidwai Memorial Institute of Oncology.

cfDNA was extracted with QIAamp Circulating Nucleic Acid Kits (Qiagen) as per the manufacturer's instructions; cfDNA was quantified using a Picogreen dsDNA quantitation kit (Life Technologies, UK).

Genotyping and analysis

Genome-wide single-nucleotide polymorphism (SNP) data were obtained using the Illumina Omni Express Exome Genotyping analyzing approximately 900,000 markers in triplicate method. Chip data were inspected using Genome Studio and subsequently exported for use with PLINK, and further annotation performed using ClinVar, 1000 Genomes, COSMIC and dbNSFP databases. We used SIFT, Polyphen2, Mutation Taster, Mutation Assessor, FATHMM, RadialSVM, and other tools to find the effect of the SNP.[7] We then filtered out any variant present at a global minor allele frequency of ≥1% in a range of publically available databases of sequence variation (1000 Genomes, COSMIC database) as well as those found in our own in-house exomes/genotyping data from individuals with related cancer (n = 200).

We applied methods based on the aforementioned approaches to detect signals of positive selection (OncodriveFM, OncodriveCLUST).[8] All mutation calls were annotated with Oncotator, Snpeff, SIFT, PolyPhen and compared against COSMIC database. We further employed the Cancer Gene Census as the most reliable catalog of known cancer genes to date to find the frequency of mutated genes across the samples.

Mutated loci were annotated using ANNOVAR. Only non-synonymous single nucleotide variants and indels in coding exons and splicing sites were included in the study.

Results

Demographic and clinicopathological data

There were 30 newly diagnosed patients with metastatic NSCLC during the study period. The median age was 60.3 years with a male:female ratio of 3.8:1. 26 patients (92%) had adenocarcinoma and four patients (8%) squamous cell cancer. 18 (69.2%) out of 26 patients with adenocarcinoma were smokers (recent or former). All the patients with squamous cell lung cancer were heavy smokers (≥25 cigarettes/day).[9] All the patients were histopathologically confirmed AJCC Stage IV [Table 1]. cfDNA data were available for 26 patients (for rest of the four patients, cfDNA could not be extracted due to very low levels in the blood).

Mutational landscape

The exome genotyping identified several somatic mutations with the highest incidence of p53 mutations (61.5%) indicating that 16 of 26 patients were positive for p53 mutations, followed by MUC1 (5.6%) and KRAS (38.4%) [Table 2 and Figure 1]. Several kinds of mutations were observed including missense, frameshift insertions and deletions and nonsense mutations [Table 3].

Discussion

Tumor genomes contain from tens to thousands of somatic mutations. However, only a few of them "drive" tumorigenesis by affecting genes drivers, which upon alteration confer selective growth advantage to tumor cells. While only few driver genes are frequently mutated in cancer, many others are altered in a small fraction of tumors. We identified non-synonymous somatic mutations in eight genes with the highest incidence of mutations observed in TP53 (65.5%), and there were several kinds of mutations such as missense, frameshift deletion, nonsense, and silent mutations. MUC16 was observed in 57.6% cases most significant and missense, frameshift insertion, and silent mutations were observed. KRAS mutations were observed in 38.4% patients with only missense type of mutations, followed by STK11 observed in 19.2% patients with missense, frameshift deletion and silent mutations. Epidermal growth factor receptor (EGFR) and Ataxia Telangiectasia Mutated (ATM) were observed in 15.3% patients

Table 1: Demographic and clinicopathological data

Characteristics	Results
M:F	3.8:1
Median age	60.3 years
Histology (%)	
Adenocarcinoma	26 (92)
Squamous cell carcinoma	4 (8)
Smokers (%)	18 (69.2)
Pleural effusion (%)	12 (46)
Bone metastasis (%)	8 (30.7)
Adrenal metastasis (%)	4 (16)
Lung metastasis (%)	3 (11.5)
Brain metastasis (%)	3 (11.5)
Liver metastasis (%)	1 (3.8)

Table 2: Incidence of somatic mutation in NSCLC

Gene	Incidence of somatic mutation *n* (%)
p53	16 (61.5)
KRAS	10 (38.4)
EGFR	4 (15.3)
MUC16	15 (57.6)
STK11	5 (19.2)
ATM	4 (15.3)
PIK3CA	1 (3.8)
RB	1 (3.8)
MET	1 (3.84)
MSH6	1 (3.8)
NF-κβ	2 (7.6)

NF-κβ: Nuclear factor-κβ, EGFR: Epidermal growth factor receptor, NSCLC: Non-small cell lung cancer

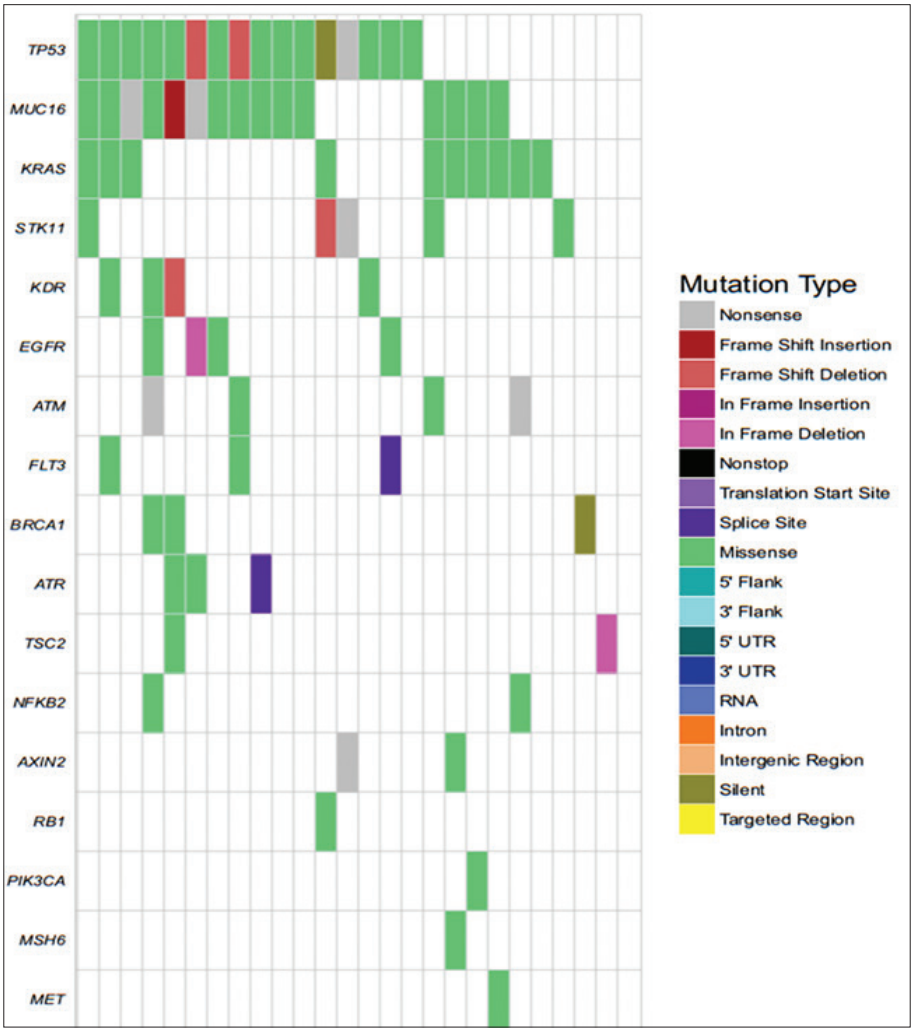

Figure 1: Represents the hierarchical cluster of mutations in non-small-cell lung cancer metastatic patients. Each column indicates 1 patient, for example, 4 patients showed mutation in KDR gene, 3 were missense mutations and 1 was frame shift deletion as indicated by the key color

Table 3: Gene mutations observed

Gene	Mutations
ATM	E574*, Q2684L
ATR	R2425Q
AXIN2	R463C
BRCA1	W1718L, E1185Q, E1038G, P871L
EGFR	T363A, G719A, L707W, del751
FLT3	N289I
KDR	Q1137K, R1232fs
KRAS	G12V, G12V, G12S, G12D, G12D, G12C, G12C, G12C
MET	X1010_splice, E608K
MSH6	D268H
MUC16	V11090A, T3788I, T6267K, G13513C, S9192G, S9147*, S2923G, R14400W, L5286fs, L7999I, G9394V, T4818S, D5535N, L4229S. L6753F, C13395F, A5005S, P14443A, A4520T, L6259Q, V2472I, T8779A, R344K
NF-κβ	A392E
PIK3CA	E418K, R88Q
STK11	X155_splice, P203L, M289I, G227fs, E120*
TP53	Y220C, X307_splice, V173L, S106R, R306*, R249M, R181C
TSC2	S315L

NF-κβ: Nuclear factor-κβ, EGFR: Epidermal growth factor receptor

with in-frame deletion and missense mutations seen in EGFR and silent and missense mutations observed in ATM. All other mutations were observed in <10% of patients.

TP53 is the most common mutation observed among all cancers, besides tumor suppressive activity due to loss of wild-type p53, many mutations in p53 lead to gain of function leading to increased migratory and invasive properties and increased resistance to therapy.[10] On the other hand, MUC16 is used as a biomarker for ovarian cancer, with recently more information on the pathological role in cancer. The C-terminal domain is associated with proliferation, migration, and resistance.[11] Dysregulation of PI3K/AKT pathway occurs through a variety of mechanisms including activation of tyrosine kinase receptors (FLT3, KDR, and fibroblast growth factor receptor) upstream of PI3K and mutations in PIK3CG, TCL1 which leads to aberrant protein synthesis and cell proliferation. Genetic alteration in STK11 gene acts as tumor suppressor controls the state of change in TP53 and regulates cell polarity and ATM that further activates CHK1/2, and CHK1/2 consequently activating TP53 in the p53 signaling pathway. However, there are a few limitations

in the pilot study such as the sample size (n = 26). A larger cohort would be able to provide a better overview of the mutational landscape of lung cancer. Follow-up of the patient could be done to understand the change in the genomic profile over a period, as well as a comparative analysis using tissue could be performed to establish the role of cfDNA in cancer testing. The use of NGS platform would be able to analyze amplifications which would provide more information on the mutational landscape of lung cancer and multivariate analysis with demographic and clinicopathological data can be performed using multiple logistic regression.

Integration of mutational profiling to identify genetic alterations is required to facilitate personalized lung cancer therapy and increase the options of treatment to decrease mortality and increase overall survival.

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