Pathology & Oncology Research (2020) 26:109–114
https://doi.org/10.1007/s12253-019-00771-5

SHORT COMMUNICATION

Spectrum of Pathogenic Germline Mutations in Chinese Lung Cancer Patients through Next-Generation Sequencing

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Received: 8 July 2019 / Accepted: 22 October 2019 / Published online: 12 November 2019

Abstract

Lung cancer is currently a leading cause of cancer-associated mortality worldwide. Despite the increasing evidences of variants that were associated with lung cancer risk, investigations of genetic factors and their roles in genetic susceptibility to lung cancer were limited. Here we systematically investigated the spectrum of pathogenic germline mutations in Chinese population with lung cancer. Genomic profiling of DNA was performed through next-generation sequencing (NGS) on tissue biopsy from 1764 Chinese lung cancer patients with a 381 cancer gene panel between January 01, 2017 and May 07, 2019. Patients with germline mutations were identified, and their clinical information were collected. Of 1764 patients with lung cancer, 67 (3.8%) patients were identified to carry pathogenic or likely pathogenic germline mutations in 25 cancer predisposition genes, with a frequency of 3.6% in lung adenocarcinoma (49/1349), 4.3% in squamous cell lung cancer (14/322), 5.6% in small cell lung cancer (4/72), and none in lung adenosquamous carcinoma (0/21), respectively. The highest pathogenic germline mutational prevalence were found in BRCA2 (0.79%), CHEK2 (0.40%), BRCA1 (0.34%), and TP53 (0.34%). Two splice mutations were reported for the first time in this study. Notably, a majority (85.5%) of the detected germline mutations fell in DNA damage repair pathways.

Keywords Germline mutation · Next-generation sequencing · Lung cancer · DNA damage repair pathway

Introduction

Lung cancer is currently the leading cause of cancer-related mortality around the world [1]. While smoking and environment pollution are the major risk factors involved in the development of lung cancer, there are multiple genetic factors playing important roles in carcinogenesis of lung. For example, mutations in genes including epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) were well-known somatic oncogenic alternations in lung cancer. However, by far, the risk factors for inherited lung cancer are poorly understood. Some studies have investigated the roles of germline alternations, mostly selected mutations, in genetic susceptibility to lung cancer [2–6], while systematic studies of the germline mutations potentially predisposing to lung cancer, especially in Chinese patients, are rarely reported. With the introduction of next-generation sequencing (NGS) technology, molecular testing has become standard in clinical practice and has changed the clinical management of lung cancer. In the present study, we showed the analyses of inherited germline mutations performed in Chinese population with lung cancer tested by a NGS panel with 381 cancer

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related genes. This study aimed to systematically investigate the spectrum of pathogenic germline mutations in Chinese lung cancer patients and determine the proportion of clinically actionable mutations detected, which will play an increasingly important role in its treatment in the future.

Material and Methods

Clinical Cancer Specimens

Formalin-Fixed Paraffin-Embedded (FFPE) tumor specimens from surgery ($N = 718$, 40.7%) or biopsy ($N = 1046$, 59.3%) of lung cancer patients were enrolled in this study. The pathological diagnosis of the specimens was confirmed by hematoxylin and eosin (H&E) staining. To be considered as a qualified sample, the specimen has to be ≥1 mm and the percentage of tumor cells should be over 20%. 50–200 ng of DNAs extracted from the samples were broke into ~200 bp fragments and then subject to NGS.

NGS Sequencing

We analyzed the sequencing data with a well-designed 381 cancer gene panel from 1764 lung cancer patients. NGS was performed on Illumina Nextseq 500 to >500X coverage as previously described [7] and was conducted in 3DMed Clinical Laboratory Inc., a College of American Pathologists (CAP) certified and Clinical Laboratory Improvement Amendments (CLIA) certified laboratory of 3D Medicines Inc. Somatic and germline alternations were identified and clinical information including age, gender, and tumor histology were collected. This study was approved by the ethics committees of The First Affiliated Hospital of Army Military Medical University (KY201992). All patients provided written informed consent. Patient identity protection was maintained throughout the study.

Variant Identification

The analysis of DNA alternations have been described in our previous publication [7]. All false positive variants were treated by our in-house auto false positives removal pipeline. The sensitivity and specificity of variant calling were above 99%. The variant frequencies were filtered by non-cancer controls from public database (including dbSNP144, ESP6500, and 1000 Genomes) for common SNPs. Germline variants were identified by comparing patient's tumor to the matching blood controls.

The interpretation of sequence variants and identification of pathogenic or very likely pathogenic mutations were performed according to a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology [8] as well as the latest literature or reports from clinical trials. Clinical actionability of mutations were identified by evidence for their roles in cancer prevention or drug sensitivity based on OncoKB (https://oncokb.org/), COSMIC (https://cancer.sanger.ac.uk/cosmic), and medical literature. Identifications of the DDR-associated genes were generated from Kyoto Encyclopedia of Genes and Genomes (KEGG) database (https://www.genome.jp/kegg/) and previous literature [9].

Results

Patient Characteristics

From January 01, 2017 and May 07, 2019, a total of 1764 cases of Chinese lung cancer patients who have undergone next-generation DNA sequencing (NGS) were included in this study, including 1349 (76.5%) with lung adenocarcinoma (ADC), 322 (18.3%) with squamous cell carcinoma (SCC), 21 (1.2%) with lung adenosquamous carcinoma (ASC), and 72 (4.1%) with small cell lung cancer (SCLC). There were 1112 (63.0%) male and 652 (37.0%) female patients. The median age was 62 (IQR range, 54 to 68) (Table 1). EGFR and ALK somatic driver mutations occur in 777 (44.0%) and 98 (5.6%) patients in the overall population, and in 736 (54.6%) and 90 (6.7%) patients in lung adenocarcinoma, respectively, which is consistent with the incidences from the previous report in Chinese population [10].

Germline Mutations of Lung Cancer Patients

Of 1764 patients with lung cancer, 67 (3.8%) patients were identified to carry 62 unique different pathogenic or very likely pathogenic germline mutations in 25 cancer predisposition genes. The incidence of patients with inherited pathogenic variants was 3.6% ($n = 49/1349$) for ADC, 4.3% ($n = 14/322$) for SCC, 5.6% (n = 4/72) for SCLC, and none for ASC

Table 1 Clinicopathologic Features of 1764 Lung Cancer Cases

Characteristics	All patients ($N = 1764$)
Age, median (IQR range)	62 (54–68)
Sex, n (%)	
Male	1112 (63.0)
Female	652 (37.0)
Histology type, n (%)	
Small cell lung cancer	72 (4.1)
Non-small cell lung cancer	1692 (95.9)
Lung adenocarcinoma	1349 (76.5)
Squamous cell carcinoma	322 (18.3)
Lung adenosquamous carcinoma	21 (1.2)

(0/21) due to the small subset. Notably, a majority (85.5%) of the detected germline pathogenic mutations fell in DNA damage repair (DDR) pathways, including BRCA2, BRCA1, BLM, PALB2, RAD50, ATM, and BAP1 in homologous recombination (HR) pathway, FANCA, FANCC, FANCD2, and FANCG in the Fanconi anemia (FA) pathway, TP53, CHEK2, and ATR in DDR checkpoint signaling pathway, MSH6 in DNA mismatch repair (MMR) pathway, and MUTYH in base excision repair (BER) pathway. The frequency of patients with at least one mutation in DDR related genes were 3.2% ($n = 57$) in the overall population, 3.0% ($n = 41$) in ADC, 3.7% ($n = 12$) in SCC, and 5.6% (n = 4) in SCLC, respectively.

Of the 62 pathogenic germline variants detected, there were 21 frameshift mutations, 22 nonsense SNVs, 15 missense SNVs, and 4 splice site mutations. In overall, 46 (68.7%) out of the 67 patients carried clinically actionable mutations. The clinical information and the distribution of all pathogenic germline mutations in the patients were shown in Table 2. These variants fell predominantly in BRCA2 ($n = 14$, 0.79%), CHEK2 ($n = 7$, 0.40%), BRCA1 ($n = 6$, 0.34%), and TP53 (n = 6, 0.34%) (Fig. 1). Amongst all, two splice mutations had never been previously reported, including BRCA2 c.8487 + 1G > C in a 51-year-old female with ADC who harbored concomitant somatic EGFR L858R mutation, and BAP1 c.38-2A > G in a 63-year-old male with ADC. The most common variant is the missense mutation of CHEK2 p.H371Y with four recurrences, followed by nonsense mutation MUTYH p.R19* with two recurrences, and the others occurred once for each. In addition, for patients carrying pathogenic germline mutations, concomitant somatic EGFR driver mutations were found in 43.3% ($n = 29$) of the patients and 57.1% ($n = 28$) in the ADC population, which is comparable to the prevalence in the overall population. Only one patient was identified with concomitant ALK mutation (EML4-ALK fusion) and pathogenic germline mutations (BLM p.R791C) (Table 2). A TMB-H (top quartile) status ($n = 350/1676$, 2.09%) or a MSI-H status ($n = 13/1710$, 0.76%) was observed in the evaluable population. One 61-year-old male with SCC with BRCA2 p.S780* mutation was identified as MSI-H among the 67 carriers of germline pathogenic mutations. No association of inherited mutations with tumor mutational burden (TMB, $P = 0.48$) or histology type ($P = 0.70$) was observed in the studied cohort.

Discussion

In this study, we identified 62 different pathogenic germline mutations in 25 genes in 67 patients from 1764 Chinese patients with lung cancer. BRCA2 c.8487 + 1G > C and BAP1 c.38-2A > G splice mutations were reported for the first time as germline mutations.

To the best of our knowledge, this is the first systematic study in pathogenic germline mutations in Chinese population with lung cancer. Previous studies for germline mutations identification in lung cancer focused primarily on limited genes or in a small subset. For example, the identification of germline mutations in driver oncogenes, including EGFR and HER2, has heightened interest in identifying germline mutations carrying a high inherited risk of lung cancer [3, 4, 11]. Another study has reported the spectrum of germline mutations in Brazilian NSCLC patients ($N = 45$) and detected a distinct pathogenic mutations and novel sequence variants when stratified by smoking status [6]. In addition, one previous study focused on the mutational status of eight DDR related genes among 555 lung adenocarcinoma cases extracted from TCGA database showed that 2.5% of the patients carried pathogenic germline mutations [5].

Among the mutated genes identified in our studied cohort, germline mutations in BRCA1/2 contributed to the majority of hereditary breast and ovarian cancer (HBOC) syndrome, which can lead to breast, ovarian, primary peritoneal, pancreatic, prostate, male breast cancer, as well as some others [12]. Besides, genes including MSH6, PALB2, TP53, ATM, CHEK2, and CDH1 were associated with hereditary ovarian, breast cancer, or gastric cancer [12]. Germline TP53 mutations were reported to be associated with a rare but highly penetrant familial cancer syndrome, the Li–Fraumeni syndrome, which may lead to a high risk for developing a variety of cancer types, including breast cancer and lung cancer [12].

Most of the detected germline mutations (85.5%) were involved in DNA damage repair (DDR) pathways. DDR pathway plays a critical role in the maintenance of genomic integrity by mediating the repair of DNA damage and regulating the cell-cycle progression. Deficiency in DDR pathway may lead to severe DNA damage, which may cause genome instability and trigger malignant transformation. As such, DDR-targeting is one of the important strategy in cancer therapy. Poly (ADP-ribose) polymerase (PARP) inhibitors (e.g. olaparib) are the best-known for its use as targeted DDR inhibitors, which exploits synthetic lethality principle to selectively kill cancer cells. PARP inhibitors have been approved in the treatment of patients harboring BRCA1/2 mutations in ovarian and breast cancer, with more trials ongoing for the others including pancreatic and prostate cancer [13]. Components targeting DDR gene mutations other than PARP were also under development [13, 14]. Given the leading incidence and mortality rate of lung cancer with a worldwide incidence of 2,100,000 in 2018 [1], the mutational prevalence of 3.3% as presented in the studied cohort represented a sizable proportion of lung cancer patients with pathogenic germline mutations in DDR pathways and more importantly, an intriguing possibility of targeting DDR proteins in this subset.

Table 2 Clinical Information for 68 Patients Carrying Pathogenic Germline Mutations

Patient No.	Sex	Age	Histology Type	Pathogenic germline Mutation	Somatic EGFR/ALK mutation
1	F	67	ADC	MUTYH R19*	EGFR E746_A750del
2	M	71	SCC	CHEK2 H371Y	
3	M	34	ADC	TP53 R158HBLM L258Efs*7	EGFR D770_N771insG EGFR gain
4	M	42	SCC	BRCA2 S1741Tfs*35	
5	M	60	SCLC	BRCA1 H318Lfs*24	
6	F	66	ADC	ATM 497-1G > C	EGFR E746_A750del
7	M	74	ADC	FANCG W451Cfs*21	
8	F	52	ADC	TP53 R248W	
9	F	72	ADC	BRCA2 Y1661*	EGFR A767_V769dup
10	M	60	ADC	FANCA Y843*	
11	M	37	ADC	BRCA1 I1824Dfs*3	
12	M	74	SCC	BRCA2 Q2530*	
13	F	52	ADC	CDKN2A V115 L	EGFR L747_P753delinsS
14	M	58	SCC	EGFR R831H	
15	F	33	ADC	FANCD2 N1378Sfs*5	EGFR L747_A750delinsP
16	F	66	ADC	CHEK2 H371Y	
17	M	44	SCC	MUTYH W153*	
18	M	71	ADC	CDH1 T340A	EGFR E746_A750delinsIP
19	M	61	SCC	BRCA2 S780*	
20	F	45	ADC	BRCA2 E1646Nfs*19	EGFR A767_V769dup
21	M	73	ADC	RAD50 Q826*	
22	M	59	SCC	TP53 P152L	
23	M	61	ADC	ATR V2125Sfs*6	
24	M	71	ADC	BRCA2 I1485Nfs*3	
25	M	62	SCC	BLM R791C	
26	M	72	ADC	BRCA2 P1145*	
27	M	64	ADC	BRCA1 E1158*	
28	M	71	SCC	BRCA2 D191V	
29	F	47	SCLC	PALB2 D161Efs*17	EGFR L747_P753delinsS
30	F	66	ADC	CHEK2 H371Y	EGFR S752_I759del EGFR gain
31	M	70	SCLC	RAD50 R1200*	
32	F	51	ADC	BRCA2 8487 + 1G > C	EGFR L858R
33	M	63	ADC	BAP1 38-2A > G	
34	F	55	ADC	CHEK2 Y139*	EGFR E746_A750del
35	F	55	ADC	BRCA1 K339Rfs*2	EGFR L858R EGFR L747_P753delinsS
36	M	55	SCC	BRCA2 D191V	
37	F	63	ADC	FANCA E605Vfs*7	EGFR L858R
38	M	29	SCC	TP53 R248Q	
Patient No.	Sex	Age	Histology Type	Germline Mutation	Somatic EGFR/ALK mutation
39	M	76	ADC	RAD50 C681*	EGFR L858R EGFR gain
40	F	36	ADC	MSH6 R1076C	
41	F	51	ADC	ATM D2672Ifs*8	
42	F	52	ADC	FANCG Y551*	EGFR E746_A750del
43	F	44	ADC	BRCA2 S2120*	
44	M	61	SCC	NF1 R1362*	
45	M	46	SCC	TP53 R196*	

Table 2 (continued)

46	M	78	ADC	JAK2 V617F	EGFR L858R
47	F	75	ADC	CHEK2 1375 + 1G > A	
48	M	71	ADC	TP53 Y220C	
49	F	65	ADC	SDHA R554Q	EGFR E746_A750del
50	F	55	ADC	BRCA2 K157Sfs*24	
51	M	65	ADC	FLCN L13Rfs*24	EGFR E746_A750del
52	F	54	ADC	TSC2 D1690Gfs*27	EGFR E746_A750del
53	M	46	ADC	BRCA2 G3134Afs*29	EGFR E709_T710delinsD
54	M	68	SCLC	BRCA1 Y1666*	
55	F	40	ADC	SDHA R554Q	EGFR E746_S752delinsV
56	F	62	ADC	FGFR3 P250T	
57	M	57	ADC	PALB2 R753*	
58	M	61	ADC	ATR N1135*	EGFR L858R
59	F	71	ADC	CHEK2 H371Y	EGFR L858R
60	M	54	SCC	BRCA1 L502Afs*2	
61	F	52	ADC	FANCD2 F465Lfs*13	
62	F	67	ADC	BRCA2 E357*	EGFR E746_A750del EGFR T790M
63	M	58	ADC	MUTYH R19*	EGFR L858R
64	F	84	ADC	ATR R1412*	EGFR E746_A750del
65	F	70	ADC	BLM R791C	EML4-ALK
66	F	68	ADC	CHEK2 S15Qfs*62 BLM Q802Gfs*12	EGFR L858R EGFR gain
67	F	61	ADC	FANCC R174*	EGFR L858R

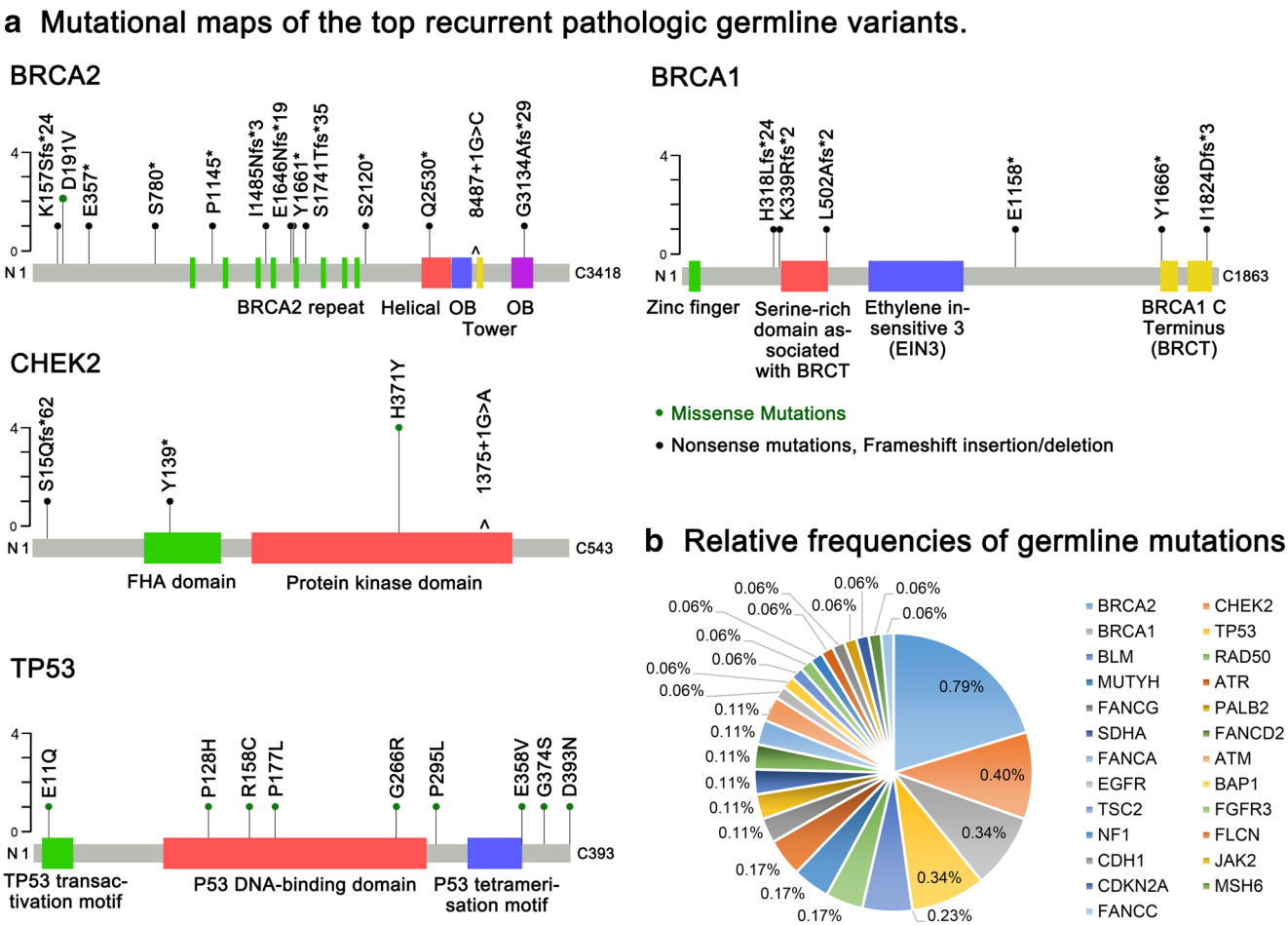

Fig. 1 Pathogenic germline variants in lung cancer patients. **a** Mutational maps of the most frequently mutations. **b** Distribution of the relative frequencies of the detected germline mutations

Taken together, we have presented the spectrum of pathogenic germline mutations in a large Chinese lung cancer cohort. The findings of deleterious inherited genetic variations may provide clues for the oncology treatment strategy and cancer prevention.

Acknowledgements Drs. Zhengyi Zhao, Yuzi Zhang, Celimuge Bao, Yanyan Wang, and Shangli Cai are employees of 3D Medicines Inc. The remaining authors declare no conflict of interests.

Author Contributions Conception and design: Panwen Tian, Xiangyang Cheng, Guowei Ma, and Ying Huang.

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Ying Huang accepts full responsibility for the work and/or the conduct of the study, had access to the data, and oversaw the decision to publish.

Compliance with ethical standards

Disclosure The author(s) received no specific funding for this work.

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