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Mutation differences in circulating tumor DNAs from non-small cell lung cancer patients between Uygur and Han populations

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Abstract

Background: The incidence of non-small cell lung cancer (NSCLC) in Uygur population is gradually increasing recently. In view of the great diagnostic and prognostic values of cell-free DNAs (cfDNA) detection, this study focus on a liquid biopsy to explore the value of cfDNA mutation in healthy and NSCLC patients in 2 ethnicities.

Methods: The concentration and sequencing of cfDNA in NSCLC and healthy subjects was assessed with a standard information analysis procedure, including detection, annotation, and statistical analysis. Gene ontology and Kyoto Encyclopedia of Genes and Genomes pathway analyses were conducted to analyze the function of mutant genes and related pathways. Totally, 133 subjects, including 35 Uygur NSCLC patients, 10 Uygur healthy subjects, 63 cases of Han NSCLC patients and 25 Han health control, were admitted to the hospital.

Results: There were a lower proportion of adenocarcinoma and higher percentage of smoking rate for Uygur patients. For cfDNA level between NSCLC and healthy subjects, Han patients exhibited sharp increase while there was no statistical difference in Uygur population. In addition, the mutation frequency of cfDNA in Han patients (72.6%) was significantly higher than Uygur patients (45.7%). There were 5 gene mutations only found in Han patients and ABCC11 showed a higher mutation frequency in Uygur population as a common one. Finally, Go and Kyoto Encyclopedia of Genes and Genomes analysis showed apprent functional enrichments and pathway changes between 2 ethnicities.

Conclusion: There existed distinct distributions of cancer subtypes, smoking proportion, cfDNA level, and mutation patterns between Han and Uygur patients. The results may be a useful tool in NSCLC patients' diagnosis as well as individualized therapy between ethnicities in future.

Abbreviations: ADC = adenocarcinoma, cfDNAs = cell-free DNAs, CNV = copy number variation, ctDNA = circulating tumor DNA, LB = liquid biopsy, NSCLCs = non-small cell lung cancers, SCC = squamous cell carcinoma, SNV = point mutations, SV = structural variation, TB = tissue biopsy.

Keywords: cell-free DNAs, circulating tumor DNA, gene mutation, non-small cell lung cancer, Uygur

1. Introduction

Lung cancer has been regarded as a leading cause of cancer death around the world.[1] Non-small cell lung cancers (NSCLCs) was the dominant type of lung cancer.[2,3] NSCLC patients are generally subjected to surgery, radiotherapy, or chemotherapy.[4] Adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are the major 2 subtypes of NSCLCs.[5] Tumor biopsy technique based detection of NSCLC subtypes has a high diagnostic and

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This study was conducted after obtaining local ethical committee approval of Shihezi University School of Medicine, the First Affiliated Hospital and written informed consent from the patients.

The authors have no conflicts of interest to disclose.

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The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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therapeutic value. Tissue biopsy (TB) has been the golden standard for NSCLC diagnosis.[6–8] TB made the accurate diagnosis possible by providing the information of tumor ADC/SCC subtype, grade/stage, and genotype probing for treatment strategy decision. However, TB has some recognized limitations. For example, patients with advanced NSCLC are often intolerable to the surgical procedures such as lung puncture; many patients even carry tumors at some sites unable to reach and obtain.[9] Besides, TB severely delays the diagnosis and treatment process, as well as weighs the economic burden of patients.[10] In addition, TBmay introduce secondary secondary infections and unpredictable inflammatory reactions. Finally, heterogeneity of tumors also leads to the limited function of TB in revealing a comprehensive genetic landscape.

Based on this, researchers have explored to obtain genetic information of tumors as early as possible through cheap and convenient means in NSCLC diagnosis. Recently, the emergence of ultra-sensitive and highly specific molecular detecting techniques has provided novel approaches for understanding the molecular features and dynamics, especially for circulating DNA in cancer patients. With the continuous improvement of Next-generation sequencing, it is possible to perform DNA detection with extremely high sensitivity. Liquid biopsy (LB), an innovative biopsy method, has interested doctors and scholars.[11–13] It offers the opportunity to monitor circulating tumor DNA (ctDNA) and circulating free DNA (cfDNA) at the whole-body system level.[14–16] Studies shows that LB based ctDNA detection currently is one of the most accurate and non-invasive method in cancer diagnosis, which showed a better sensitivity and accuracy better than imaging.[17–19]

Different cultural customs, dietary habits, genetic background, and regional distribution lead to differences in cancer onset. In western China, Han and Uygur are 2 dominant nations. The incidence of lung cancer in the Uygur population is gradually increasing recently,[1] although lung cancer in the Han population is significantly higher than that in Uighurs. Considering of the great diagnostic and prognostic values of cfDNA detection, this study aims to reveal the level and mutation difference between Uighur and Han patients with NSCLC to explore the diagnosis and therapeutic values of cfDNA.

2. Materials and methods

2.1. Patients

This study was approved by the hospital ethics committee and was performed between December 1, 2016 and November 3, 2017. The subjects in this study lived in Shihezi and Kashgar areas of Xinjiang Province. Patients with advanced NSCLC were admitted to the First Affiliated Hospital of Shihezi University School of Medicine and Kashi District Hospital. Totally, we enrolled 133 subjects: 35 Uygur NSCLC patients (median age 56, 23–84), 10 Uygur normal population (median age 56, 23–84), 25 Han population health control (median age 57, 33–84) and 63 cases of Han NSCLC patients (median age 57, 33–84).[20–22] The blind method was used for selection. In terms of living habits, Uygur people eat more meat, eat more milk and dairy products, eat less vegetables, mainly eat beef and mutton, do not eat pork, and rarely eat fish. Han people eat more balanced, and they eat all kinds of meat and vegetables.[23–24]

The inclusion criteria of patients were as follow:

(1) Diagnosed as NSCLC by histopathology or cytology;
(2) Han or Uygur patients;
(3) Patients who have not undergone surgery with complete clinical data;
(4) Patients who did not receive radiotherapy, chemotherapy, or targeted drugs before blood sampling;
(5) Age: 18 to 85 years old;
(6) The indexes of blood, urine, feces and heart, liver, kidney, and lung function were normal.

The exclusion criteria were as follow:

(1) Diagnosed as Small cell lung cancer by histopathology or cytology;
(2) Patients with secondary NSCLC;
(3) Patients who had received chemotherapy, radiotherapy, and targeted therapy just 1 week prior to blood sampling;
(4) Patients with heart, liver, kidney, lung function disorders;
(5) The clinical data are incomplete.

Correspondingly, the criteria for inclusion of healthy controls were as follows:

(1) Age: 18 to 85 years old;
(2) The indexes of blood, urine, feces and heart, liver, kidney, and lung function were normal.

2.2. Blood sample collection

Peripheral blood was collected from each patient. The collection procedure was performed only after the consent was signed by each patient (or the family members). A volume of 10 mL blood was collected into the anticoagulant tube, and the plasma separation was completed within 4 hours.

Briefly, the plasma separation process was as follow: the tube was centrifuged (1600 g for 10 minutes) at 4°C, and the upper layer was dispensed into a plurality of 1.5 mL tubes. The separated plasma sample was centrifuged again (16000 g for 10 minutes) at 4°C. The supernatant was transferred to a new tube and stored at –80°C. Besides, the middle leukocyte layer was aspirated as control use.

2.3. DNA extraction

The cell-free DNAs (cfDNAs) were extracted from the plasma samples. An appropriate amount of sample (3 mL) was added to the 15 mL centrifuge tube, and then 300 μL proteinase K was added to the tube and mixed by vortex for 15 seconds. Afterwards, 2.4 mL Buffer ACL was added and mixed by vortex for 30 seconds. The tube was incubated at 60°C for 30 minutes. Next, 5.6 mL carrier RNA and 5.4 mL buffer ACB were added, and DNA was extracted through the QIAamp Mini Nucleic Acid Extraction Column in a QIAvac 24 Plus system in accordance with the official standard protocol. After extraction process, the collected DNA was labeled, and later, DNA electrophoresis was used to detect the DNA quality.

2.4. DNA quality and concentration detection

The plasma-extracted DNA was subjected to quality and concentration detection using the Qubit Fluorometer. DNA samples were mixed with 2.5 μL 6 × bromophenol yellow and added to each well. The total amount of DNA in each well was 50 to 100 ng. Agarose gel electrophoresis was performed at a voltage

of 130 V for 30 minutes. The quality of DNA was assessed according to the banding condition of the electrophoresis, which was observed under the UV lamp. A clear and complete band meant a better DNA quality; while a severe tailing indicated the DNA was broken or degraded. The concentration of cfDNA extracted from plasma was detected with qubit. The cfDNA concentration of every patients (including Uygur, Han and healthy subjects) were listed in table S1 (see Table S1, Supplemental Content, which illustrates the concentration of cfDNA in Uyger patient and healthy group, http://links.lww.com/MD/F544) and table S2 (see Table S2, Supplemental Content, which illustrates the concentration of cfDNA in Han patient and healthy group, http://links.lww.com/MD/F545). Unpaired *t* test was performed among the 3 group.

2.5. DNA sequencing

The starting input of nucleic acids was not less than 20ng. The Illumina Hiseq 3000 system was used for sequencing, and 1021 genes were captured after sequencing. Raw reads were obtained, filtered, and compared to the reference genomes for decontamination, and unique mapped reads aligned to the genome were obtained. Standard information analysis procedures were performed, including detection, annotation, and statistical analysis. The original data was aligned to the HG37 reference genome sequence for analysis of the point mutations (SNV), Indel, copy number variation (CNV), and structural variation (SV) using the Kayinga Autonomous Analysis Protocol.

2.6. TMB ratio analysis

TMB = the number of mutations per 1MB coding region. TMB was normalized by dividing the total mutations by the coding region (1.0 Mb) captured in the panel. The criterion of TMB-H: ≥10 mutations/Mb. Fisher exact test was used to compare proportions between 2 groups. The mutation status of all patients was listed in table S3 (see Table S3a–c, Supplemental Content, which illustrates the TMB-H distribution in Uygur and Han NSCLC patients, the mutation spectrum in all Uygur and Han patients, and the EGFR mutation status in Uygur and Han patients, http://links.lww.com/MD/F546).

2.7. Statistical analysis

Results obtained were compared between 2 nations or groups (Uygur vs Han, or patients vs health control). The frequency comparison was performed using the Fisher exact analysis, and the continuous quantitative data was compared by the *t* test analysis. For all the presented data, $P < .05$ was considered statistically significant.

3. Results

3.1. Patient characteristics

As shown in Table 1 & Table 2, 24 (68.6%) were ADC cases and 7 (20.0%) squamous were cell carcinoma cases; besides, there were 2 cases (5.7%) of large cell neuroendocrine tumor, 1 (2.9%) case of adenoid cystic carcinoma and 1 case unknown. The stage-III patients accounted for 14.3% (5 cases), and stage-IV patients accounted for 85.7% (30 cases). The patients with a history of smoking accounted for 20.0% (7 cases) and non-smoking ones accounted for 80.0% (28 cases). Sixty-three Han NSCLC patients were enrolled, including 58 (92.1%) ADC patients, 4 (6.3%) SCC patients and 1 (1.6%) patient was unknown in subtype determination. Four (6.3%) patients and 59 (93.7%) patients at Stage-III patients and stage-IV respectively. Only 2 (3.2%) patients with smoking history in Han NSCLC patients. It is noteworthy that the proportion of ADC in Uygur population was lower than that of Han population (68.6 vs 92.1, Fisher exact test, $P = .0041$); and the proportion of smokers was significantly higher in Uygur than the Han population (Fisher exact test, $P = .0093$).

Table 1

Uygur population characteristics.

	Case	Percentage	Mutant	Non-mutant
Total	35		16	19
Sex				
Male	22	62.9%	12	10
Female	13	37.1%	4	9
Age	56 (23–84)		60 (43–84)	53 (23–71)
Subtype				
ADC	24	68.6%	10	14
SCC	7	20.0%	4	3
Other	4	11.4%	2	2
Stage				
III	5	14.3%	2	3
IV	30	85.7%	14	16
Smoke				
Yes	7	20.0%	4	3
No	28	80.0%	12	16

3.2. Uygur patients had higher cfDNA level than Han patients

CfDNA concentration was determined for each subject. The average plasma cfDNA level in Uygur NSCLC patients was 29.9 ng/mL (6.7–129.8 ng/mL), and 32.1 ng/mL (8.9–99.2 ng/mL) respectively (Table 3). There was no significant difference between NSCLC and healthy subjects in Uygur population, However, Uygur inhabitants showed a dramatically higher basal cfDNA concentration compared with Han inhabitants (Fig. 1 A & C). The average cfDNA concentration in Han

Table 2

Han population characteristics.

	Case	Percentage	Mutant	Non-mutant
Total	63		47	16
Sex				
Male	32	50.8%	23	9
Female	31	49.2%	24	7
Age	57 (33–84)		57 (33–84)	57 (36–82)
Subtype				
ADC	58	92.1%	43	15
SCC	4	6.3%	3	1
Other	1	1.6%	1	0
Stage				
III	4	6.3%	1	3
IV	59	93.7%	46	13
Smoke				
Yes	2	3.2%	1	1
No	61	96.8%	46	15

Table 3

The cell-free DNAs concentration in patients and healthy subjects.

Variables	Patient number	cf DNA range(ng/mL)	Average cfDNA (ng/mL)
Uygur patient	35	6.7–129.8	29.9
Uygur healthy	10	8.9–99.2	32.1
Han patient	47	4.7–71.9	21.9
Han healthy	25	3.4–8.4	5.4

cfDNA = cell-free DNAs.

NSCLC patients was 21.9 ng/mL (4.7–71.9 ng/mL) (Table 3), which was highly significantly up-regulated compared with the 5.4 ng/mL (3.4–8.4 ng/mL) in healthy Han population controls ($P < .0001$) (Fig. 1D). In addition, Uygur NSCLC patients had higher plasma cfDNA concentration than Han NSCLC patients ($P = .0473$) (Fig. 1 B), although cfDNA concentration was enhanced to nearly 4 times of the baseline in Han patients. This sharp increase was not found in the Uygur nation (Fig. 1C). This difference might be caused by different ethnic genetic backgrounds, dietary conditions, and other factors in Uygur and Han populations. Together, Uygur and Han population had different cfDNA levels and exhibited distinct change response to NSCLC occurrence.

3.3. Mutation differences between Uygur and Han populations

In healthy subjects (25 Han and 10 Uygur cases), not any mutation was detected.

As shown in Figure 2A, 16 of 35 (46%) Uygur patients were found mutation carriers. A total of 131 SNVs or Indel, 2 CNVs, and no SVs of 87 genes were detected. Four of them (25%) had high mutational burden (TMB-H, more than 9 mutations); 2 patients had copy number gain (CNG). Nine (56.3%) patients carried TP53 mutations, 8 (50.0%) had EGFR mutations and 4 (25.0%) patients had LRP1B mutations. ABCC11 and

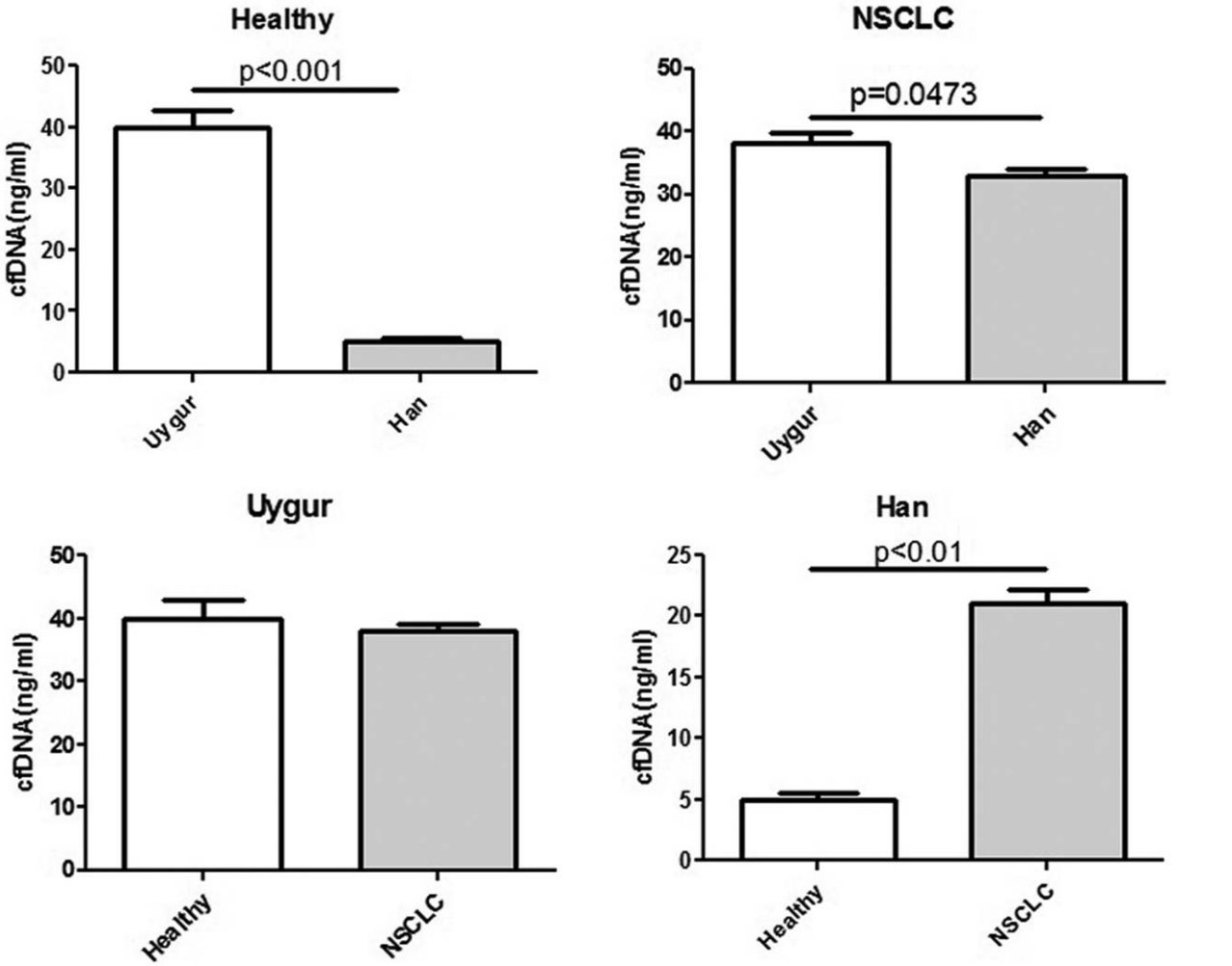

Figure 1. The concentration of plasma cell-free DNAs (cfDNAs) in healthy and non-small cell lung cancer (NSCLC) patients in 2 ethnicities. (A) The cfDNA concentration of healthy Uygur and Han subjects. (B) The cfDNA concentration of Uygur and Han NSCLC subjects. (C) The cfDNA concentration in healthy and NSCLC subjects in Uygur population. (D) The cfDNA concentration in healthy and NSCLC subjects in Han population. Uygur cfDNA concentration is significantly higher than Han population among healthy subjects ($P < .001$), and slightly higher among NSCLC patients ($P < .05$). There has no statistic difference for cfDNA concentration between Uygur patients and healthy ones ($P > .05$). Han patients show a dramatically increase of cfDNA concentration compared to healthy subjects ($P < .01$). Unpaired *t* tests were performed among different groups.

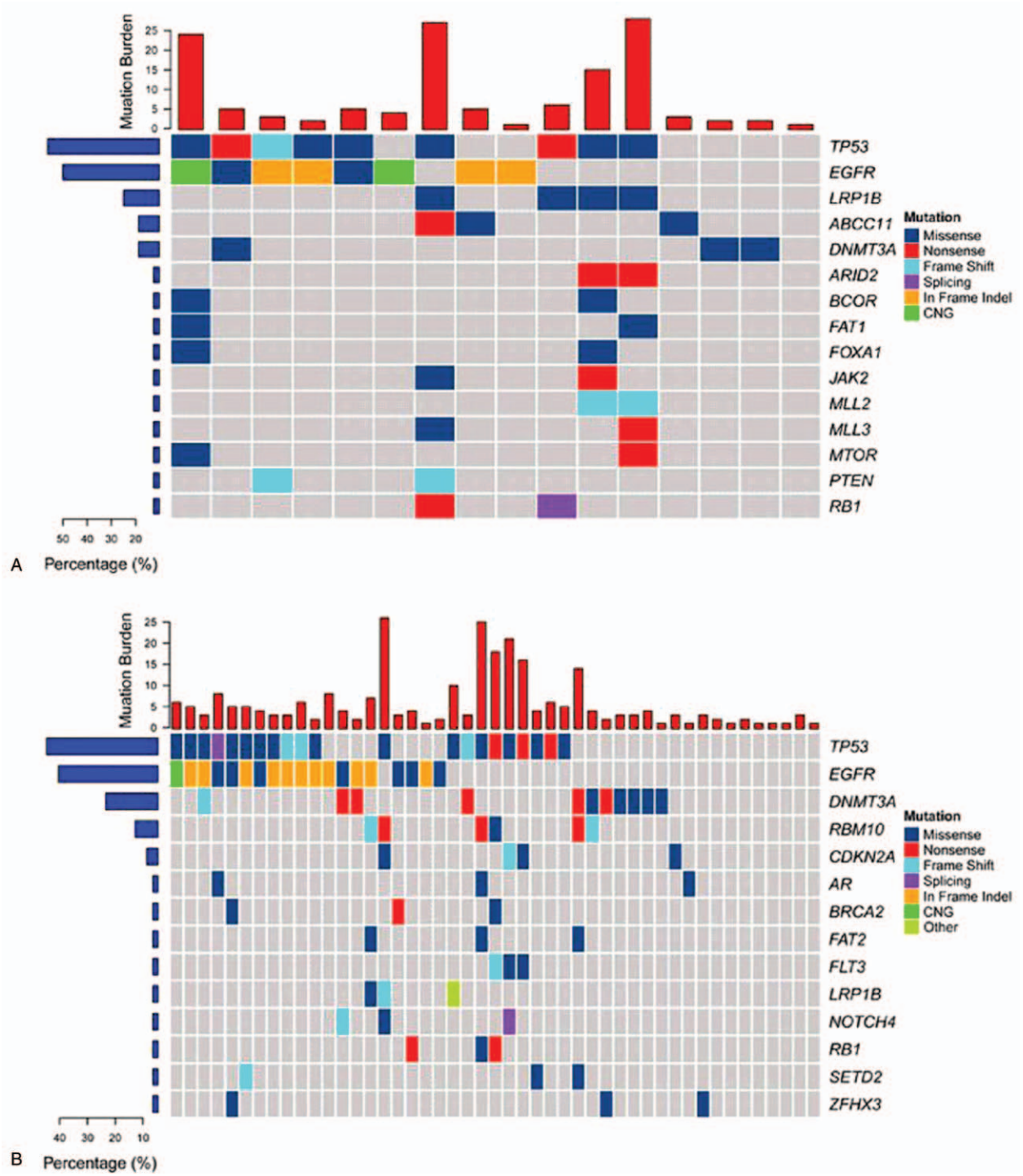

Figure 2. Mutation spectrum of Uygur and Han patients including the point mutations, Indel, copy number variation, and structural variation. The top frequently common mutations in Uygur patients (A) and Han patients (B) are listed. Among 35 Uygur patients, 16 cases carried mutations, 4 cases had high tumor mutation burden and the top frequently common mutations are listed. Among 63 Han patients, 47 cases carried mutations, 7 cases had high tumor mutation burden and the top frequently common mutations are listed. Different mutation patterns are labeled in different colors.

DNMT3A mutations appeared in 18.8% cases, and PTEN and RB1 mutations appeared in 12.5% cases. Only 1 patients were detected KRAS (p.G12D) mutation.

Forty-seven (74.6%) Han patients were found to carry mutations (Fig. 2B). A total of 260 SNVs or Indels, 2 CNVs, and 3 SVs of 147 genes were found. The 94 patients had an average of 6 SNVs or Indels, with the minimum of 1 and the maximum of 26. Seven of them (14.9%) were TMB-H carriers; 2 (4.2%)patients had CNG of EGFR or ERBB2. Among the 47 patients, 21 (44.7%) had TP53 gene mutations, 19 (40.4%) had EGFR gene mutations, 6 (12.8%) had RBM10 gene mutations. Moreover, CDKN2A, LRP1B, RB1, and NOTCH4 mutations accounted for 6.4% cases. Similarly, 2 patient was observed KRAS (p.G12D) mutation.

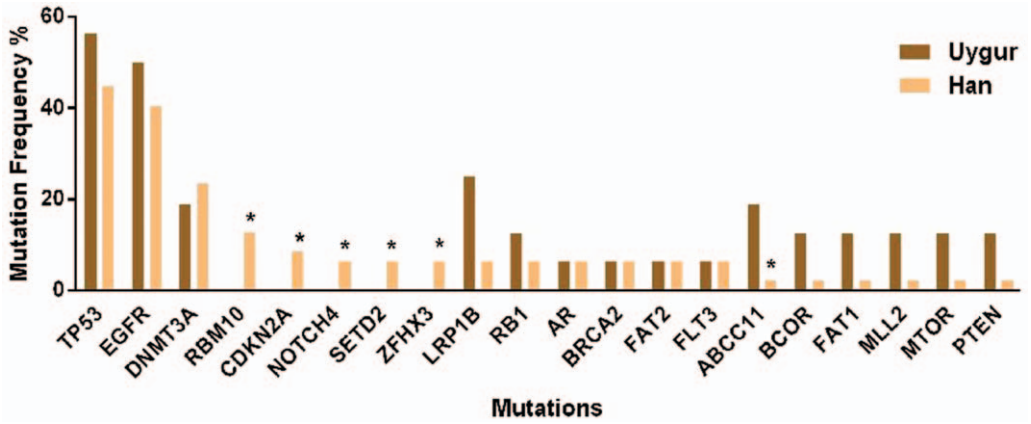

Figure 3. Comparison of the top mutations between 2 ethnicities. There was no significant difference in mutation burden between 2 nations ($P > .05$) and 5 mutations (RBM10, CDKN2A, NOTCH4, SETD3, ZFHX3) were found only in Han patients. As a common mutation, ABCC11 was significantly higher in Uygur population compared to Han population ($P < .05$). Unpaired t tests were performed between Uygur and Han patients.

Incomparison, the mutation ratio in Han patients (72.6%, 47/63) was significantly higher than Uyghur patients (45.7%, 16/35) (Fisher exact test, $P = .0077$). And in the mutation-positive patients, there was no significant difference in mutation burden between 2 nations ($P = .2008$). p53 and EGFR were the most common mutations. And some gene mutations (RBM10, CDKN2A, NOTCH4, SETD3, ZFHX3) were only found in Han patients but not in Uyghur ones (Fig. 3). A common mutation, ABCC11, was significantly higher in the Uygur population compared with Han population (18.8% vs 2.1%, Fisher exact test, $P = .04724$). The ABCC11 gene-encoded protein (also called MRP8) is a multidrug resistance-associated protein, which is an efflux pump of nucleotide analogs and 5-fluoro-2'-deoxyuridine 5'-phosphate (FdUMP), which can enhance active metabolites. FdUMP is effluxed to confer 5-FU resistance. indicating the stronger chemotherapy resistance in Uygur group. There were no significant differences in other genes between the 2 nations.

Among the 16 Uygur patients with mutations, 2 cases showed CNV (amplification) of the EGFR gene. In the 47 Han patients with mutations, 1 (2.1%) had EGFR amplification and 1 (2.1%) had ERBB2 gene amplification. The CNV frequency were 12.5% (2/16) and 4.3% (2/47) in the Uygur and Han populations, respectively; no significant difference between the nations were found (Fisher exact test, $P = .1561$). Among the Uygur patients, no SV was found; but 3 (6.4%) of the Han patients carried SV (CD74-ROS1, EML4-ALK, and KIF5B-RET, respectively). However, there was no statistical significance (Fisher exact test, $P = .5645$) (Fig. 3).

Further, we analyzed the high tumor mutation burden (TMB-H) cases of each nation. The TMB-H ratio in 2 nations were 25.0% (Uygur) and 14.9% (Han), respectively (Fisher test, $P = .4485$) (see Table S3a, Supplemental Content, which illustrates the TMB-H distribution in Uygur and Han NSCLC patients, http://links.lww.com/MD/F546). In the Uygur TMB-H patients, 3 were smokers and 1 was non-smokers. In contrast, none of the 7 Han patients with TMB-H was smoker. The proportion of smokers in Uygur TMB-H patients was significantly higher than that of Han patients (Fisher exact test, $P = 0.0242$).

3.4. Gene ontology and Kyoto encyclopedia of genes and genomes analysis

Through gene ontology enrichment analysis (Fig. 4 A&B), the differences in the function of mutant genes were analyzed. Uygur patients' mutant genes were involved in 37 molecular functions, and the Han patients' mutant genes were involved in 60 molecular functions. Among them, there were 5 types of functions (inositol phosphate phosphatase activity, thyroid hormone receptor binding, phosphatidylinositol phosphate phosphatase activity, disordered domain specific binding, RNA polymerase II transcription coactivator activity, 3'-5'-exodeoxyribonuclease activity) were found in Uygur population but not in Han population. On the other hand, 29 functionalities (presented by the protein serine/threonine kinase activity) were found in the Han patients but not in Uygur population. Kyoto Encyclopedia of Genes and Genomes signaling pathway analysis(Fig. 5 A & B) showed a significant enrichment of Wnt pathway in Uygur patients but not in Han patients. Besides, mTOR, Notch, AMPK, VEGF and other pathways were found enriched in Han population but not the Uygur population. Together, above data implied that the different mutations of cfDNA in Uygur and Han patients may provide precise therapeutic targets and prognosis biomarker to NSCLC patients of Uygur and Han patients.

4. Discussion

The main findings of the study include:

(1) Higher percentage of Uygur NSCLC patients had smoking history and ADC rate is significantly lower in Uygur patients compared with Han patients;
(2) Han patients experienced sharp increase of cfDNA after NSCLC occurrence while no change of that were observed in Uygur patients. In addition, the plasma cfDNA content was significantly higher in Uygur patients compared with the Han nationality;
(3) Han patients (63.6%) had higher mutation frequency than Uygur patients (45.7%);
(4) Among top mutations, RBM10, CDKN2A, NOTCH4, SETD3, ZFHX3 were only found in Han patients but not

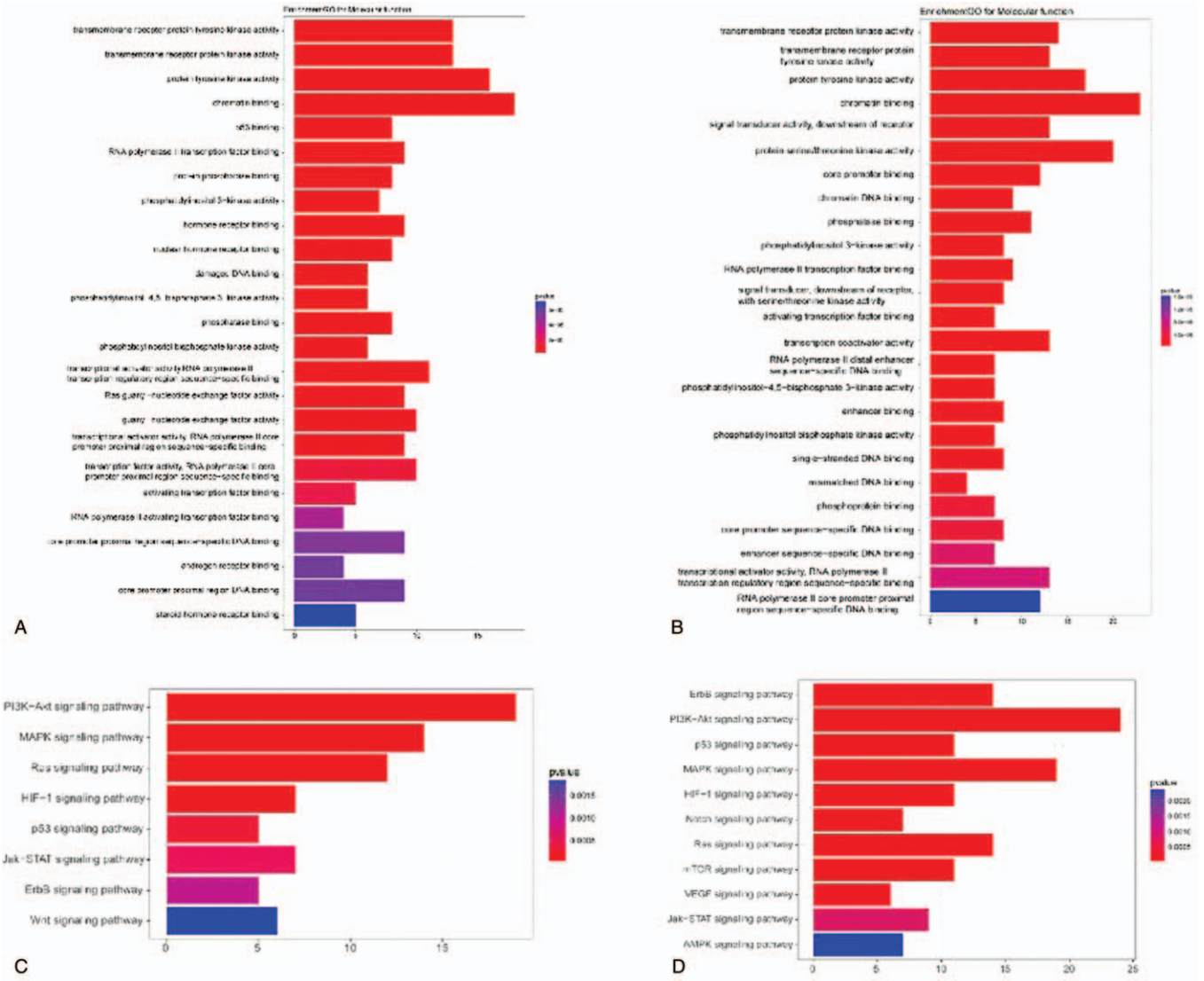

Figure 4. Gene ontology (GO) analysis of mutant genes in Uygur and Han patients. (A) GO enrichment analysis of Uygur patients' mutant genes involved in 37 molecular functions. Among them, there were 5 types of functions found only in Uygur population. (B) GO enrichment analysis of Han patients' mutant genes involved in 60 molecular functions. Among them, there were 29 types of functions found only in Han patients.

Uyghur ones. Among the common mutation, ABCC11 had a higher frequency in Uygur;

(5) Smokers among the TMB-H patients were also more frequent in Uygur patients than Han patients;

(6) Go analysis showed 5 types of functional mutant genes only in Uygur patients and other 29 types of functional mutant genes only in Han population; Wnt pathway was significantly enriched in Uygur patients while mTOR, Notch, AMPK, and VEGF pathways were enriched in Han patients.

LB techniques based on ctDNA or cfDNA have unique clinical significance in capturing tumor progression, guiding accurate treatment of individuals, and improving patient outcomes.[25–27] Compared to the TB, this method allows repeating sampling and continuous monitoring for real-time observation of changes. Another potential advantage of ctDNA or cfDNA analysis is the ability to determine the combined genomic content of all clones from all disease sites in the context of heterologous for multifocal cancers,[25] as shown in our study. Another superiority is that, besides blood samples, some researchers have also isolated ctDNA from urine.[28,29] Urine-derived ctDNA detection increased progression-free survival and overall survival by 0.44 and 0.35 months; and urine-2d ctDNA approaches avoid potential complications induced by TB and eliminating approximately 55.6% of theTB-based molecular tests, through which each patient can save 1243 to 1680 dollars. Together, LB based detection may be a useful, convenient and economical tool in cancer diagnosis.

Historically, the Uyghurs are a united nation with a commonality with the Han, Arab, and Caucasian populations in phenotypes. They have both characteristics like Han and Caucasian in the Rh blood groups,[30] and their CR1 gene A4646G that decides the Knops blood group has both the characteristics of Han and Caucasian populations.[31] Also, in genetic polymorphism and differential expression, there are many reported differences between Uygur and Han ethnicities. Some

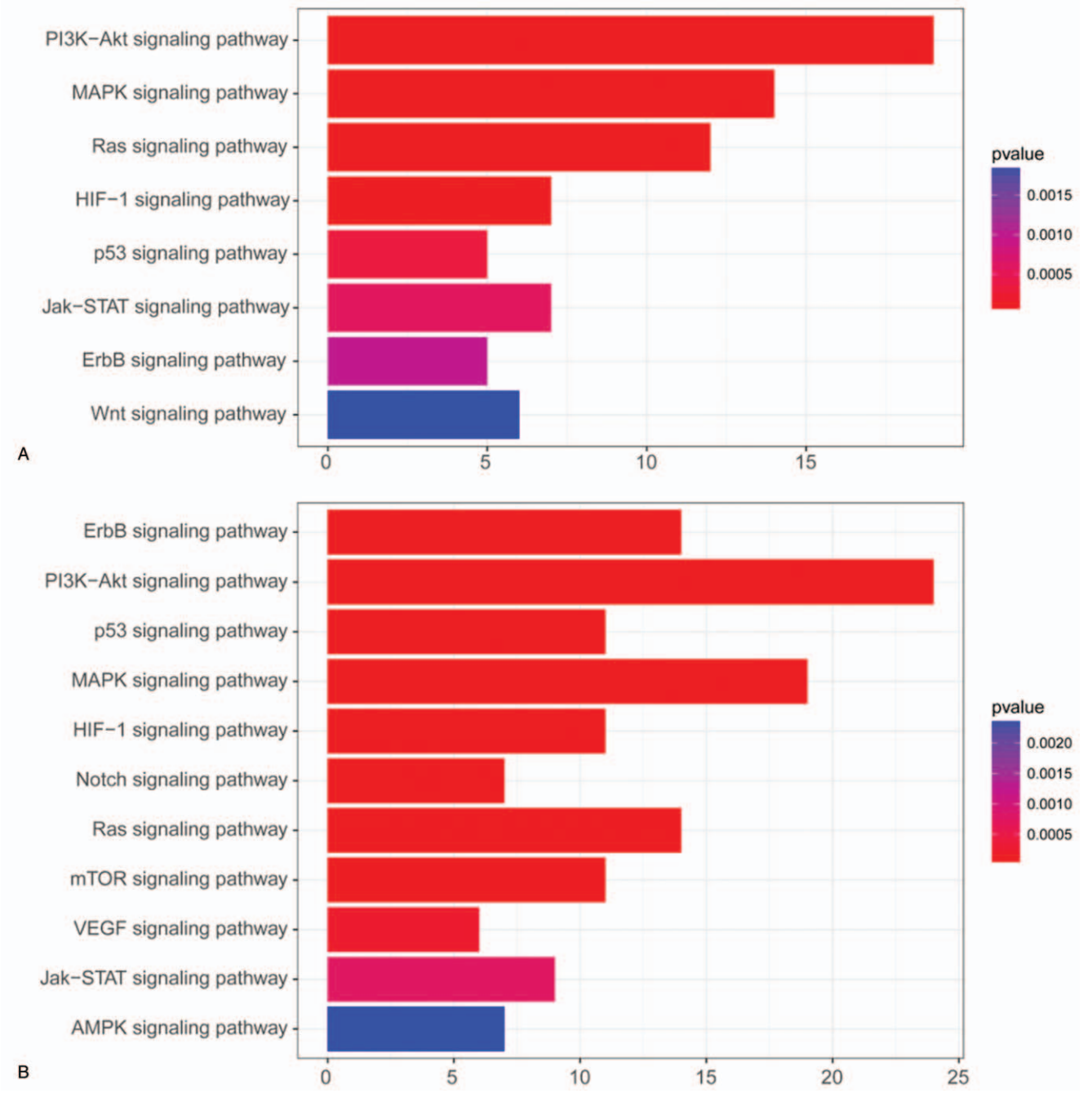

Figure 5. Kyoto Encyclopedia of Genes and Genomes signaling pathway analysis of mutant genes in Uygur (A) and Han (B) patients. Wnt pathway activation was predicted only in Uygur patients but not in Han patients. And mTOR, Notch, AMPK, VEGF and other pathways were found enriched only in Han patients.

widely known differences in gene polymorphism are as follow: the cytochromes P4502C19 gene polymorphisms,[32] GJB2 35delG carrier rate in deafness patients,[33,34] p53 Arg72Pro polymorphisms in cervical cancer patients,[35,36] the angiotensin-converting enzyme (ACE) gene insertion/deletion (I/D) differences which impacted essential hypertension,[37] differences in caries susceptibility which were related to the HLA-DQB1 allele,[38] the KCNE1 gene as an independent risk factor for atrial fibrillation in the Uygur population but not in Han,[39] and so on. However, there are not sufficient data regarding to genetic differences in NSCLC between the 2 nations. Limited findings in the field of cancer research were acquired majorly in breast cancer and cervical cancer: the expression levels of ER-b, ER-a and Her-2 are different between the Uygur and Han patients[40,41]; and several studies focused on some risk gene expression in Uygur women with cervical cancer but did not mention the data of Han patients.[42,43] So far, the present study is the first one that reveals mutation differences between these 2 ethnicities.

Some interesting findings of our study are that Uygur smokers accounted more in NSCLC patients than Han, especially in TMB-H patients, while ADC patients accounted fewer. To date, there has been no statistical data about the smoking proportion

difference between Uygur and Han population, let alone the levels in lung cancer. One study mentioned a ratio of 24.7% NSCLC patients had the history of smoking,[44] which was similar to our statistics. Some Chinese researches reported that increased exposure to cigarettes might induce ADC onset (but towards the Han population).[45,46] Generally, this effect works in both ADC and SCC types,[47–50] but it seems to promote SCC more than ADC.[51–56] The mechanisms may include miR-101–3p/COX-2 pathway, SSBP2 promoter methylation, and Wnt signaling, etc. In addition, another possible reason of the higher Uygur smoking ratio accompanied with higher SCC proportion is that smoking may promote the trans-differentiation of ADC towards SCC through upregulation of Notch3, Wnt/β-catenin and TGF-b pathways.[57–59] Combining our results of signal pathway enrichment, that Uygur patients had significantly enriched Wnt pathway but not Han patients, we strongly supported the Wnt pathway may play a role in determining the high SCC proportion in Uygur smokers. Based on above data, it is reasonable to infer that Uygur NSCLC patients may have a higher risk of cancer and have a worse prognosis as well as a higher probability of drug resistance. This inference can be supported by independent published data. For example, a study of esophageal SCC pointed out that the tumors in Uygur patients showed more resistance to radiotherapy and chemotherapy than Han patients, and radiotherapy/chemotherapy resulted in longer survival of Han patients.[60] We can speculate that the in vivo condition of the Uygur patient confers a more suitable microenvironment for the occurrence, survival, and proliferation of SCCs. In addition, Uygur children in Xinjiang have higher obesity frequency than other Chinese ethnicities.[61] Moreover, Uygur women have a high proportion of BRCA1 mutation and incidence of breast cancer,[62,63] which is consistent with the different ABCC11 mutation rate in our observation.

Theoretically, the mutation detection rate and mutation burden of ctDNA are effective predictors of a high malignancy and poor prognosis. The mutation rate in Han patients was significantly higher than that in Uygur patients, but there was no significant difference in mutation burden among the positive individuals. The results suggest that the Uygur population may have higher resistance to NSCLC onset, but the Han population may have a higher risk to develop NSCLC once mutations occur.

This study has some limitations. For example, according to smoking, we have not collect the information about smoking intensity and duration, which might be closely associated with mutations. Besides, our work did not further investigate the association between cfDNA mutations, tumor phenotypes and patient prognosis.

5. Conclusion

We conducted cfDNA sequencing in Uygur and Han patients and found that

(1) The cfDNA level experienced sharp increase after NSCLC occurrence in Han patients but not in Uygur patients;
(2) The plasma cfDNA content of Uygur patients was higher than Han patients, but their overall mutation frequency was lower than Han population.
(3) Compared to the Han population, the Uygur population has a significant Wnt pathway enrichment, and 5 types of functional mutations represented were found only in the Uygur population.
(4) The detection of cfDNA level, mutation difference and gene enrichment may be a useful tools in NSCLC patients diagnosis as well as individualized therapy between ethnicities.

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