Identification of genome variations in patients with lung adenocarcinoma using whole genome re-sequencing

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Abstract. Lung adenocarcinoma is one of the types of non-small cell lung carcinoma, which tends to be treated with surgical therapy rather than radiation therapy. It occurs in smokers and non-smokers, and is the most common form of lung cancer among non-smokers and women. Gene rearrangements, including ALK, ROS1 and RET, and gene mutations, including epidermal growth factor receptor (EGFR), HER2, Kristen rat sarcoma viral oncogene homolog, BRAF, phosphoinositide-3-kinase, catalytic, α polypeptide and MET, have been identified in lung adenocarcinoma, which enable targeted therapy in lung adenocarcinoma, for example erlotinib, gefitinib and afatinib, which are EGFR inhibitors. The aim of the present study was to further investigate genome variations in lung adenocarcinoma. Single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), structural variations (SVs) and copy number variations (CNVs) were identified in the whole genome from four patients with adenocarcinoma using a whole genome re-sequencing method performed on the Illumina HiSeq Xten platform. In total, ~415 GB of clean reads were obtained, the average sequencing depth was 31.10-fold, and 99.29% of the reference genome was covered by the clean reads. An average of 3,364,270 SNPs was identified, 98.76% of which were matched to the SNP database (dbSNP), and an average of 453,547 InDels were identified, 99.29% of which were matched to the SNP database. In the present study, ~8% of lung cancer cases are caused by genetic factors (7). Genetic and environmental factors can damage DNA to alter the epigenetics, which affects the normal functions of cells, including DNA repair, cell proliferation and apoptosis (8). There have been numerous studies investigating the association between gene variation and lung adenocarcinoma. The epidermal growth factor receptor and Kristen rat sarcoma viral oncogene homolog have been identified as mutations in lung adenocarcinoma as important driver genes (9). In addition, single nucleotide polymorphisms (SNPs) have been found to be associated with lung cancer, including colony-stimulating factor 1 receptor, tumor protein p63 and co-repressor interacting with RBPJ1 (10). Successful mapping of the human genome and the emergence of next-generation sequencing technology have assisted in the identification of specific variations associated with disease comprising SNPs, insertions and deletions (InDels), structure variations (SVs) and copy number variations (CNVs). This has already assisted in understanding and investigating several diseases, including melanoma (11), lung cancer (12), breast cancer (13) and acute myelogenous leukemia (14), and has also assisted in intergenic regions and coding sequence regions. In conclusion, the results of the present study generated an output of the genome alterations in lung adenocarcinoma, and provided a foundation for further investigation of the pathogenesis of lung adenocarcinoma.

Introduction

Lung cancer is a type of malignant tumor, which is one of the most life-threatening to humans. The morbidity and mortality rates in men with lung cancer are the highest of all types of malignancy, and lung cancer has the second highest following breast cancer in women (1). Smoking is the major cause of lung cancer (2) and accounts for 85% of lung cancer cases in the world (3). Lung cancer includes small-cell lung carcinoma and non-small-cell lung carcinoma (NSCLC) (4), and lung adenocarcinoma is a type of NSCLC, which is found in peripheral lung tissue (1). It is the most common type of lung cancer in smokers and in those who have never smoked (5). Lung adenocarcinoma grows slowly and usually forms small masses, however, they readily metastasize at the early stage (6).

Cancer is caused by genetic and environmental factors, and ~8% of lung cancer cases are caused by genetic factors (7). Genetic and environmental factors can damage DNA to alter the epigenetics, which affects the normal functions of cells, including DNA repair, cell proliferation and apoptosis (8). There have been numerous studies investigating the association between gene variation and lung adenocarcinoma. The epidermal growth factor receptor and Kristen rat sarcoma viral oncogene homolog have been identified as mutations in lung adenocarcinoma as important driver genes (9). In addition, single nucleotide polymorphisms (SNPs) have been found to be associated with lung cancer, including colony-stimulating factor 1 receptor, tumor protein p63 and co-repressor interacting with RBPJ1 (10). Successful mapping of the human genome and the emergence of next-generation sequencing technology have assisted in the identification of specific variations associated with disease comprising SNPs, insertions and deletions (InDels), structure variations (SVs) and copy number variations (CNVs). This has already assisted in understanding and investigating several diseases, including melanoma (11), lung cancer (12), breast cancer (13) and acute myelogenous leukemia (14), and has also assisted in...
in disease diagnosis, screening of drug targets and prediction of disease risk.

In the present study, the genome variations of four tumor tissues from different patients with lung adenocarcinoma were detected, using a whole genome re-sequencing method performed on the Illumina HiSeq Xten platform. From this, the SNPs, InDels, SVs and CNVs from these four samples were identified, and the SNPs and InDels were annotated, respectively.

**Patients and methods**

**Patient samples and DNA extraction.** Tumor samples were collected from four patients with lung adenocarcinoma. The four patients were all diagnosed with lung adenocarcinoma using pathological methods and were classified to have stage IV tumors, as bony metastases had occurred prior to diagnosis. All were treated with chemotherapy only (Table I). Written informed consent was obtained from all patients. The study was approved by the ethics committee of Shanghai Tongji Hospital (Shanghai, China). Genomic DNA was extracted from tumor samples using standard phenol/chloroform extraction methods (15). Agarose gel electrophoresis was used to confirm that the DNA samples were not degraded and that RNA was not contaminated. The quality and quantity of DNA were assessed using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Inc.), following which the DNA samples with optical density values between 1.8 and 2.0, and a content >1.5 µg were used for library construction.

**Library construction and sequencing.** The present study was performed by Beijing Novogene Bioinformatics Technology Co., Ltd, Beijing, China (www.novogene.com/). Briefly, the qualified DNA samples were randomly sheared into DNA fragment sizes of 350 bp using the Covaris S220 Focused Ultrasonicator (Covaris, Woburn, MA, USA), and library construction was performed according to the manufacturer’s protocol of the TruSeqDNA Library Construction kit (Illumina, Inc., San Diego, CA, USA). The Qubit® 2.0 Fluorometer was used first for preliminary quantification following the completion of library construction, following which the Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Waldbronn, Germany) was used for determining the insert size of the library. This was followed by accurate quantification using the quantitative polymerase chain reaction method to ensure the library effective concentration was ≥2 nM. Sequencing was performed using the Illumina HiSeq Xten platform (Illumina, Inc.) to obtain the raw reads. Reads with adapter sequences or of low quality were filtered out to obtain clean reads, which were used in the following analysis.

**Read mapping and identification of SNPs, InDels, SVs and CNVs.** The high quality filtered reads were mapped to the reference genome UCSC hg19 (16) using BWA 0.7.8-r455 software (17). The initial alignment results underwent duplicate removal, local realignment and base quality recalibration processing using Picard 1.111 (sourceforge.net/projects/picard/), GATK v3.1 (software. broadinstitute.org/gatk/) (18) and SAMTools 1.0 (samtools.sourceforge.net) (19), respectively. The effective data were evaluated for the sequence read depth and coverage of the clean reads mapping to the reference genome. SAMtools 1.0 software (19) was used to identify SNPs and InDels (fragment size of insertion or deletion <50 bp), estimate the accuracy of SNP data with the transition/transversion ratio (ts/tv), with the whole genome ratio being ~2.2 (20), and matching these SNPs and InDels to the SNP database (dbSNP) (www.ncbi.nlm.nih.gov/projects/SNP/) (21). The identification of SVs was performed using Breakdancer 1.4.4 software (genome.ustl.edu/tools/cancer-genomics/) (22), containing large fragments of deletions, insertions, duplication and copy number variants, inversion and translocation. CNVs were identified using Control-FREEC v6.7 software (bioinfo.curie.fr/projects/freec/tutorial.html) (23), containing deletions and duplications.

**Results**

**Genome sequencing and mapping to the reference genome hg19.** Genome DNA was extracted from tumor tissues of four patients with lung adenocarcinoma and genome sequencing was performed with the Illumina HiSeq Xten platform. A total of 415.98 G raw data was generated on 25 paired-end lanes, and an average of 693,316,202 raw reads were obtained for each sample, clean reads accounted for ~99.88% of the original data. A Phred quality score is a measure of the quality of the identification of the nucleobases generated by automated DNA sequencing. Phred quality scores Q are defined as a property which is logarithmically related to the base-calling error probabilities P, Q=-10 log_{10} P (24). The proportion of four bases was 86-91% when the Phred score was >30, amongst which GC content accounted for 40.92-43.71% (Table II).

The numbers of filtered clean reads were 620,492,220, 694,563,450, 679,993,278 and 774,771,688 for YJY, GMY, ZCG and JLY2, respectively, were properly mapped to the reference genome of UCSC hg19. The average sequencing depths were 28.31-31.08-, 30.55- and 34.47-fold, and 99.66, 98.96, 99.62, 98.93 of the reference genomes were covered by the clean reads, respectively. Coverage at least 4-fold were 99.32, 98.69, 99.19 and 98.70%, respectively (Table III). As shown in Fig. 1A, the association between the ratio of bases in each sample and the sequence depth was in accordance with the Poisson distribution. The mean depths and coverage of each chromosome of the reference genome are shown in Fig. 1B, showing a mean depth of ~47 and coverage of ~97%, with the exception of sex chromosomes.

**Detection of SNPs and InDels.** The detection of SNPs and InDels were performed using SAMTools, and matched to the dbSNP. There were 3,346,792, 3,387,147, 3,334,068 and 3,389,071 SNPs, respectively for YJY, GMY, ZCG and JLY2, of which 96.38, 97.20, 97.71 and 97.25%, respectively, were properly mapped to the reference genome are shown in Table I. The initial alignment results underwent duplicate removal, local realignment and base quality recalibration processing using Picard 1.111 (sourceforge.net/projects/picard/), GATK v3.1 (software. broadinstitute.org/gatk/) (18) and SAMTools 1.0 (samtools.sourceforge.net) (19), respectively. The effective data were evaluated for the sequence read depth and coverage of the clean reads mapping to the reference genome. SAMtools 1.0 software (19) was used to identify SNPs and InDels (fragment size of insertion or deletion <50 bp), estimate the accuracy of SNP data with the transition/transversion ratio (ts/tv), with the whole genome ratio being ~2.2 (20), and matching these SNPs and InDels to the SNP database (dbSNP) (www.ncbi.nlm.nih.gov/projects/SNP/) (21). The identification of SVs was performed using Breakdancer 1.4.4 software (genome.ustl.edu/tools/cancer-genomics/) (22), containing large fragments of deletions, insertions, duplication and copy number variants, inversion and translocation. CNVs were identified using Control-FREEC v6.7 software (bioinfo.curie.fr/projects/freec/tutorial.html) (23), containing deletions and duplications.
Similarly, of the InDels, ~6% were located in exonic regions (24,304 in 443,118, 2,5246 in 461,393, 23,806 in 436,058, 25,672 in 473,617), an average of 647 InDels were located in the CDS, ~12% (75, 73, 78, and 74) were identified as frame shift deletions, and 9% (54, 55, 59, and 55) were frame shift insertions (Fig. 3).

Annotation of SNPs and InDels. The ts/tv ratios of all samples were ~2.2 (YJY, 2.11; GMY, 2.10; ZCG, 2.11; JLY2, 2.10), the numbers of TS and TV types of SNPs were 2,269,999 and 1,076,793, 2,296,037 and 1,091,110, 2,263,437 and 1,070,631, 2,297,128 and 1,091,943 for each sample. Compared with the dbSNP, an average of 98.76% of the SNP sites were matched and an average of 41,563 SNPs were novel in each sequence data (40,762, 41,619, 41,694, 42,176; Table IV). For the InDels, there were 443,118, 461,393, 436,058 and 473,617 in YJY, GMY, ZCG and JLY2, respectively, the majority of which were heterozygote and homozygote; ~28.28% (126,222, 130,260, 124,274 and 132,201) were found in the dbSNP, and the remaining were novel (Table V).

Table I. Patient information.

| Patient | Sex | Age (years) | Cancer | Stage | Tumor sample |
|---------|-----|-------------|--------|-------|--------------|
| YJY     | Female | 67          | Lung adenocarcinoma | IV    | Primary tumor |
| GMY     | Female | 75          | Lung adenocarcinoma | IV    | Primary tumor |
| ZCG     | Male   | 65          | Lung adenocarcinoma | IV    | Primary tumor |
| JLY2    | Male   | 52          | Lung adenocarcinoma | IV    | Primary tumor |

Table II. Quality of sequencing data.

| Sample | Raw reads (n) | Raw data (G) | Clean reads (n) | Effective (%) | Q20 (%) | Q30 (%) | GC (%) |
|--------|---------------|--------------|----------------|---------------|---------|---------|--------|
| YJY    | 621,362,127   | 93.20        | 620,492,220    | 99.86         | 96.27; 93.27 | 90.88; 85.32 | 42.05; 41.99 |
| GMY    | 695,467,557   | 104.32       | 694,563,450    | 99.87         | 96.53; 93.07 | 91.35; 86.04 | 40.93; 40.93 |
| ZCG    | 680,810,250   | 102.12       | 679,993,278    | 99.88         | 96.43; 94.08 | 91.26; 86.94 | 43.71; 43.66 |
| JLY2   | 775,624,875   | 116.34       | 774,771,688    | 99.89         | 96.55; 93.63 | 91.38; 86.22 | 40.95; 40.92 |

Table III. Summary of sequenced reads aligned to the reference genome of hg19.

| Sample | YJY | GMY | ZCG | JLY2 |
|--------|-----|-----|-----|------|
| Total  | 620,492,220 (100%) | 694,563,450 (100%) | 677,993,278 (100%) | 774,771,688 (100%) |
| Duplicate | 62,418,141 (10.10%) | 83,850,807 (12.12%) | 78,946,241 (11.66%) | 98,027,674 (12.70%) |
| Mapped | 617,778,824 (99.56%) | 691,799,013 (99.60%) | 677,069,702 (99.57%) | 771,860,901 (99.62%) |
| Properly mapped | 598,003,088 (96.38%) | 675,101,840 (97.20%) | 664,436,740 (97.71%) | 753,442,342 (97.25%) |
| PE mapped | 615,682,036 (99.22%) | 689,578,896 (99.28%) | 674,885,134 (99.25%) | 769,555,296 (99.33%) |
| SE mapped | 4,211,636 (0.68%) | 4,440,234 (0.64%) | 4,369,136 (0.64%) | 4,611,210 (0.60%) |
| With mate mapped to a different chromosome | 3,958,804 (0.64%) | 2,711,738 (0.39%) | 3,465,530 (0.51%) | 2,738,078 (0.35%) |
| With mate mapped to a different chromosome [mapQ ≥5] | 2,839,756 (0.46%) | 1,788,585 (0.26%) | 2,347,772 (0.35%) | 1,691,563 (0.22%) |
| Average sequencing depth | 28.31 | 31.08 | 30.55 | 34.47 |
| Coverage | 99.66% | 98.96% | 99.62% | 98.93% |
| Coverage at least 4X | 99.32% | 98.69% | 99.19% | 98.70% |
| Coverage at least 10X | 97.00% | 97.93% | 96.07% | 98.15% |
| Coverage at least 20X | 79.91% | 91.11% | 76.60% | 94.19% |

PE, paired-ended reads; SE, single-ended reads; mapQ, map quality which is the effective reading criteria.

Similarly, of the InDels, ~6% were located in exonic regions (24,304 in 443,118, 2,5246 in 461,393, 23,806 in 436,058, 25,672 in 473,617), an average of 647 InDels were located in the CDS, ~12% (75, 73, 78, and 74) were identified as frame shift deletions, and 9% (54, 55, 59, and 55) were frame shift insertions (Fig. 3).

Annotation of SNPs and InDels. The ts/tv ratios of all samples were ~2.2 (YJY, 2.11; GMY, 2.10; ZCG, 2.11; JLY2, 2.10), the numbers of TS and TV types of SNPs were 2,269,999 and 1,076,793, 2,296,037 and 1,091,110, 2,263,437 and 1,070,631, 2,297,128 and 1,091,943 for each sample. Compared with the dbSNP, an average of 98.76% of the SNP sites were matched and an average of 41,563 SNPs were novel in each sequence data (40,762, 41,619, 41,694, 42,176; Table IV). For the InDels, there were 443,118, 461,393, 436,058 and 473,617 in YJY, GMY, ZCG and JLY2, respectively, the majority of which were heterozygote and homozygote; ~28.28% (126,222, 130,260, 124,274 and 132,201) were found in the dbSNP, and the remaining were novel (Table V).
Analysis of SVs and CNVs. SV identification was performed using Breakdancer 1.4.4 software, the results showed that the largest number of structural variations were deletions with average of 2,422 variations (YJY, 2,193; GMY, 2,390; ZCG, 2,220 and JLY2, 2,886). The majority of the variations were located at intergenic (~57%) and intron (~35%) regions. By contrast, the least common structural variation was inversions, with an average of 138 (YJY, 150; GMY, 130; ZCG, 133; JLY2, 138), which were predominantly located in the intergenic, intron and CDS regions (Table VI). CNVs were identified using Control-FREEC v6.7 software, which contained deletions and duplications, the majority of which were in intergenic regions.
The frequency of deletion occurrence was higher, compared with that of duplication occurrence in the four samples (Table VII).

**Discussion**

The characteristics of tumor cells include infinite proliferation and growth, evasion of the body's immune surveillance, energy metabolism on the basis of glycolysis metabolism, dedifferentiation, and invasion or migration in a clone growth manner. These biological characteristics of tumor cells differ from the basic features and evolution processes of normal cells, and are considered a result of decisive factors and genetics. Therefore, the investigation of cancer from the perspective of molecular genetics has become the mainstream. A study by Boveri (25) suggested that cancer was caused by abnormal

(92, 174, 98 and 159) and CDS regions (41, 35, 43 and 42). The frequency of deletion occurrence was higher, compared with that of duplication occurrence in the four samples (Table VII).

**Table V.** Statistics of insertions and deletions for high quality reads from YJY, GMY, ZCG and JLY2 mapped onto the reference genome of hg19.

| Sample   | YJY     | GMY     | ZCG     | JLY2     |
|----------|---------|---------|---------|---------|
| Total    | 443,118 | 461,393 | 436,058 | 473,617 |
| Heterozygote | 191,092 | 202,768 | 187,294 | 207,839 |
| Homozygote | 252,026 | 258,625 | 248,764 | 265,778 |
| dbSNP percentage | 126,222 (28.48%) | 130,260 (28.23%) | 124,274 (28.50%) | 132,201 (27.91%) |
| Novel    | 316,896 | 331,133 | 311,784 | 341,416 |

**dbSNP, single nucleotide polymorphism database.**

![Figure 2. Detection results of single nucleotide polymorphisms in each sample. (A) YJY; (B) GMY; (C) ZCG; and (D) JLY2.](image-url)
genetic material, and that the damage and induced mutation of DNA leading to cancer also indicated the importance of genetic material in cancer. Rather than explaining the role of single genes or single mutations in cancer cells, investigations are now primarily aimed at clarifying the nature of carcinogenesis through identifying whole genome variation. This includes the identification of novel mutation sites and gene mutations associated with tumors, the molecular regulatory network and the cellular signaling pathways, which these mutations are involved in, for determining the cause of cancer from the gene variation profile (26). Several cancer genes and pathways have been identified in cancer genome investigations, including the stromal antigen 2 mutation in transitional cell carcinoma of the bladder (27), FAT atypical cadherin 1, FAT atypical cadherin 2 and zinc finger protein 750 mutations in esophageal squamous cell carcinoma (28) and AT-rich interactive domain-1A, vascular cell adhesion molecule 1 and cyclin-dependent kinase 14 mutations in liver cancer (29).

In the present study, re-sequencing of four tumor genomes was performed from different patients with lung adenocarcinoma, and these sequence data were aligned to reference genome hg19 to obtain information regarding the numbers of SNPs, InDels, SVs and CNVs in each sample, respectively. The alignment results showed that changes of base ratio with sequence depth in each sample were in accordance with the Poisson distribution, and this suggested that the sequencing results were of high quality and coverage.

SNPs can occur anywhere in the genome, including CDS, untranslated region, splicing, non-coding RNA and intergenic regions; they can occur as a synonymous SNP, missense SNP, stopgain and stoploss in the CDS region, which can lead to errors in the amino acid sequence of proteins or in the regulation of translation, thus affecting cell features and function. In the present study, an average of 3,364,269 SNPs was detected in each sample, of which 98.77% were matched to the dbSNP. The ts/tv ratios were all ~2.1, indicating a high level of accuracy of the SNP data (whole genome ratio is ~2.2). InDels of the
Table VI. Statistics of structural variations for high quality reads from YJY, GMY, ZCG and JLY2 mapped onto the reference genome of hg19.

| Sample | VarType   | Total | CDS | Splicing | UTR5 | UTR3 | Intron | Upstream | Downstream | ncRNA | Intergenic | Unknown |
|--------|-----------|-------|-----|----------|------|------|--------|----------|------------|-------|------------|---------|
| YJY    | Insertion | 295   | 5   | 0        | 1    | 1    | 114    | 2        | 3          | 16    | 153        | 0       |
|        | Inversion | 150   | 33  | 0        | 0    | 0    | 32     | 1        | 0          | 12    | 72         | 0       |
|        | Deletion  | 2,193 | 49  | 2        | 2    | 5    | 748    | 12       | 20         | 79    | 1,276      | 0       |
|        | Translocation | 298 | 3   | 0        | 1    | 4    | 83     | 3        | 0          | 14    | 190        | 0       |
| GMY    | Inversion | 130   | 30  | 0        | 0    | 0    | 24     | 1        | 0          | 13    | 62         | 0       |
|        | Deletion  | 2,390 | 51  | 0        | 2    | 4    | 809    | 14       | 15         | 92    | 1,403      | 0       |
|        | Insertion | 176   | 4   | 0        | 0    | 1    | 71     | 3        | 3          | 8     | 86         | 0       |
|        | Translocation | 300 | 1   | 0        | 0    | 10   | 92     | 3        | 0          | 17    | 177        | 0       |
| ZCG    | Deletion  | 2,220 | 52  | 2        | 3    | 5    | 807    | 24       | 20         | 77    | 1,230      | 0       |
|        | Inversion | 133   | 49  | 0        | 0    | 0    | 29     | 1        | 1          | 11    | 42         | 0       |
|        | Insertion | 376   | 7   | 0        | 1    | 0    | 156    | 1        | 4          | 19    | 188        | 0       |
|        | Translocation | 378 | 4   | 0        | 1    | 5    | 110    | 3        | 0          | 19    | 236        | 0       |
| JLY2   | Deletion  | 2,886 | 57  | 1        | 3    | 8    | 1,002  | 13       | 24         | 94    | 1,684      | 0       |
|        | Insertion | 631   | 9   | 0        | 1    | 2    | 243    | 6        | 7          | 28    | 335        | 0       |
|        | Inversion | 138   | 29  | 0        | 1    | 0    | 36     | 1        | 0          | 10    | 61         | 0       |
|        | Translocation | 356 | 3   | 0        | 1    | 6    | 101    | 2        | 0          | 18    | 225        | 0       |

VarType, type of variation; CDS, coding sequence; UTR, untranslated region; ncRNA, non-coding RNA.
CDS region and splice site are likely to alter protein translation. Frameshift mutations, in which the base lengths of insertion or deletions are not a multiple of three bases, may lead to changes in the reading frame. The present study also identified numerous InDels in each sample, the majority of which were located in intergenic regions and noncoding regions, and a large proportion of InDels (~82%) were novel. However, changes in frame coding proteins, including frameshift deletions, frameshift insertions, stopgain and stoploss were present in CDS regions.

SV is widespread in the human genome, and is the source of individual differences and susceptibility to certain diseases. SV also exists in cancer cells, compared with the genome of normal tissue cells and may lead to the occurrence of fusion genes, which may be associated with cancer (30). The results of the present study showed that the most common SV type was deletions, with an average of 2,422 variations. CNV may be an important cause of certain diseases. Deletions and duplications at the chromosome level have become a focus of investigations of several diseases. The results of the present study indicated that deletions and duplications were present in all four samples, predominantly in intergenic regions (92, 174, 98 and 159) and CDS regions (41, 35, 43 and 42).

In conclusion, the present study described the genome re-sequencing results of four patients with lung adenocarcinoma and the alignment results of these sequence data. The SNPs, InDels, SVs and CNVs of each sample were identified, which aligned to the reference genome of hg19, and a simple annotation of SNPs and InDels was performed. Increasing evidence indicates that genetic variation is closely associated with diseases, including cancer. SNPs, InDels, SVs and CNVs may affect gene expression or signaling pathways, which may lead to changes in cell viability and metastasis. The occurrence and progression of lung adenocarcinoma is a complicated process with specific gene expression profile and gene functions, which are the result of genetic variation and/or environmental factors. The results of the present study showed that investigating genome variation in patients with lung adenocarcinoma assists in understanding the mechanism of lung adenocarcinoma oncogenesis. More samples and investigations of specific genetic analysis and functional annotations are required to further examine of the associations between gene variation and lung adenocarcinoma.

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References

1. Stewart BW and Wild C: International Agency for Research on Cancer and World Health Organization: World cancer report, 2014.
2. Biesalski HK, Bueno de Mesquita B, Chesson A, Chytil F, Grimble R, Herrmus R, Köhrle J, Lotan R, Norpoth K, Pastorino U and Thurnham D: European consensus statement on lung cancer: Risk factors and prevention. Lung Cancer Panel. CA Cancer J Clin 48: 164-176, 1998.
3. Yu YH, Liao CC, Hsu WH, Chen HJ, Liao WC, Muo CH, Sung FC and Chen CY: Increased lung cancer risk among patients with pulmonary tuberculosis: A population cohort study. J Thorac Oncol 6: 32-37, 2011.
4. DeVesa SS, Bray F, Vizcaino AP and Parkin DM: International lung cancer trends by histologic type: Male: Female differences diminishing and adenocarcinoma rates rising. Int J Cancer 117: 294-299, 2005.
5. Travis WD: World Health Organization, International Agency for Research on Cancer., International Association for the Study of Lung Cancer. And International Academy of Pathology.: Pathology and genetics of tumours of the lung, pleura, thymus and heart. IARC Press Oxford University Press (distributor), LyonOxford, 2004.
6. Kumar V and Robbins SL: Robbins basic pathology. Saunders/Elsevier, Philadelphia, PA, 2007.
7. Yang IA, Holloway JW and Fong KM: Genetic susceptibility to lung cancer and co-morbidities. J Thorac Dis 5 (Suppl 5): S454-S462, 2013.
8. Hong WK: American Association for Cancer Research: Holland Frei cancer medicine 8. People’s Medical Pub. House, Shelton, Conn, 2010.
9. Yatabe Y, Koga T, Mitsudomi T and Takahashi T: CK20 expression, CDX2 expression, K-ras mutation and goblet cell morphology in a subset of lung adenocarcinomas. J Pathol 203: 645-652, 2004.
10. Kang HG, Lee SY, Jeon HS, Choi YY, Kim S, Lee WK, Lee HC, Choi JE, Bae EY, Yoo SS, et al: A functional polymorphism in CSF1R gene is a novel susceptibility marker for lung cancer among never-smoking females. J Thorac Oncol 9: 1647-1655, 2014.
11. Pleasance ED, Cheetham RK, Stephens PJ, McBride DJ, Humphray SJ, Greenman CD, Varela I, Lin ML, Ordóñez GR, Bignell GR, et al: A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 463: 191-196, 2010.
12. Lee W, Jiang Z, Liu J, Haverty PM, Guan Y, Stinson J, Yue P, Zhang Y, Pant KP, Bhatt D, et al: The mutation spectrum revealed by paired genome sequences from a lung cancer patient. Nature 465: 473-477, 2010.
13. Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A, Delaney A, Gelmon K, Guliany R, Senz J, et al: Mutational evolution in alobular breast tumour profiled at single nucleotide resolution. Nature 461: 809-813, 2009.
14. Ley TJ, Mardis ER, Ding L, Fulton B, McLellan MD, Chen K, Dooling D, Dunford-Shore BH, McGrath S, Hickenbotham M, et al: DNA sequencing of a cytogenetically normal acute myeloid leukaemia genome. Nature 456: 66-72, 2008.
15. Mamiatis T, Fritsch EF, Sambrook J and Engel J: Molecular cloning2. A laboratory manual. New York Cold Harbor Laboratory. 1982. ISBN 45 S., 42 Volume 5, Issue 1. Acta Biotechnologica 5: 104-104, 1985.
16. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zehler AM and Haussler D: The human genome browser at UCSC. Genome Res 12: 996-1006, 2002.
17. Li H and Durbin R: Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754-1760, 2009.
18. DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, et al: A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 43: 491-498, 2011.
19. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G and Durbin R: 1000 Genome Project Data Processing Subgroup: The Sequence Alignment/Map format and SAMtools. Bioinformatics 25: 2078-2079, 2009.
20. 1000 Genomes Project Consortium, Abecasis GR, Auton A, Brooks LD, DePristo MA, Durbin RM, Handsaker RE, Kang HM, Marth GT and McVean GA: An integrated map of genetic variation from 1,092 human genomes. Nature 491: 56-65, 2012.
21. Sherry ST, Ward MH, Kholodov M, Baker J, Phan L, Smigielski EM and Sirotkin K: dbSNP: The NCBI database of genetic variation. Nucleic Acids Res 29: 308-311, 2001.
22. Chen K, Wallis JW, McLellan MD, Larson DE, Kalicki JM, Poli WS, McGrath SD, Wendl MC, Zhang Q, Locke DP, et al: BreakDancer: An algorithm for high-resolution mapping of genomic structural variation. Nat Methods 6: 677-681, 2009.
23. Boeva V, Popova T, Bleakley K, Chiche P, Cappo J, Schleiermacher G, Janoueix-Lerosey I, Delattre O and Barillot E: Control-FREEC: A tool for assessing copy number and allelic content using next-generation sequencing data. Bioinformatics 28: 423-425, 2012.
24. Ewing B and Green P: Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8: 186-194, 1998.
25. Boveri T: Concerning the origin of malignant tumours by Theodor Boveri. Translated and annotated by Henry Harris. J Cell Sci 121 (Suppl 1): S1-S84, 2008.
26. Garraway LA and Lander ES: Lessons from the cancer genome. Cell 153: 17-37, 2013.
27. Yan JX, Xu J, Gu ZH, Pan CM, Lu G, Shen Y, Shi JY, Zhu YM, Tang L, Zhang XW, et al: Exome sequencing identifies somatic mutations of DNA methyltransferase gene DNMT3A in acute monocyctic leukaemia. Nat Genet 43: 309-315, 2011.
28. Song Y, Li L, Ou Y, Gao Z, Li E, Li X, Zhang W, Wang J, Xu L, Zhou Y, et al: Identification of genomic alterations in oesophageal squamous cell cancer. Nature 509: 91-95, 2014.
29. Jiang H, Deng Q, Wang Q, Li KY, Dai JH, Li N, Zhu ZD, Zhou B, Liu XY, Liu RF, et al: Exome sequencing of hepatitis B virus-associated hepatocellular carcinoma. Nat Genet 44: 1117-1121, 2012.
30. Shao D, Lin Y, Liu J, Wan L, Liu Z, Cheng S, Fei L, Deng R, Wang J, Chen X, et al: A targeted next-generation sequencing method for identifying clinically relevant mutation profiles in lung adenocarcinoma. Sci Rep 6: 22338, 2016.