Original Article

Identification of somatic copy number variations in plasma cell free DNA correlating with intrinsic resistances to EGFR targeted therapy in T790M negative non-small cell lung cancer

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Background: About 20–30% EGFR-mutant non-small lung cancer show intrinsic resistance to EGFR targeted therapies. Compared to T790M positive in acquired resistance patients, little is known about EGFR-TKI intrinsic resistance for T790M negative patients.

Methods: Thirty-one patients with advanced stage lung cancer, including 18 patients with intrinsic resistance (PFS <6 months) and 13 patients with acquired resistance (PFS >36 months) but are negative for plasma T790M were recruited in the study. Plasma cell free DNA was profiled by low coverage whole genome sequencing with median genome coverage of 1.86X by Illumina X10. Sequencing coverage across chromosomes was summarized by samtools, and normalized by segmentation analysis as provided by R package 'DNACopy'.

Results: The most frequent chromosomal changes were found on chr7, chr1 and chr8. Among them, chr7p gains were found in 12 (66.7%) intrinsic resistance and 4 (30.7%) acquired resistance patients. The gene EGFR was found located on the focal amplification peak of chr7p. The performance of 7p gain to predict intrinsic resistance reaches AUC =0.902. Similarly, focal amplifications were also found on chromosome 5, 16 and 22, where tumor related gene PCDHA®, ADAMTS18 and CRKL were located. Focal deletions were also found in chr1, 8, 10 and 16, where genes SFTPA1/2, DLC1, PTEN and CDH1 are located.

Conclusions: The results suggest cell free DNA copy number might be a useful peripheral blood tumor biomarker for predicting intrinsic resistance of EGFR targeted therapy and prognosis.

Keywords: Copy number variations (CNV); plasma cell free DNA (cfDNA); intrinsic resistances; T790M negative; non-small cell lung cancer (NSCLC)

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Introduction

Lung cancer is the leading cause of cancer death worldwide. In the past decades, significant improvements have been made for lung cancer treatment, based on discoveries of molecular biomarkers and disease progression mechanisms. A landmark of these improvements is epidermal growth factor receptor (EGFR) targeting therapy for patients with advanced lung cancer bearing EGFR activating mutations (1,2). However, clinical outcomes for lung cancer still remains unsatisfactory, with 5-year survival rate of less than 20% (3).

Clinical studies have revealed that lung cancer with activating EGFR mutations that are initially responsive to EGFR-tyrosine kinase inhibitors (EGFR-TKIs) will develop acquired resistance to TKI after a median progression-free survival (PFS) of 10–16 months (2,4). On the other hand, 20–30% of patients with non-small cell lung cancer (NSCLC) harboring activating EGFR mutation show no objective tumor regression to initial EGFR-TKI treatment, and this subgroup of NSCLC has been defined as with intrinsic or primary resistance to EGFR-TKIs.

Studies have revealed that the secondary T790M mutation of EGFR is the major cause of the acquired EGFR-TKI resistance in lung cancer (5). The third-generation of EGFR-TKI, aiming targeting the acquired EGFR T790M resistance mutation, has thus been developed. Clinical studies showed that osimertinib, a first third-generation of EGFR-TKI received FDA and EMA approval, demonstrated significant clinical effects with 70% objective response rate and 10 months progression free survival in metastatic EGFR-mutant NSCLC patients failure to first-generation EGFR-TKIs (6). Other causes for the acquired EGFR-TKI resistance in lung cancer include c-MET amplification (7), HER2 and PIK3CA mutation (8).

Very little is known about intrinsic resistance of EGFR-TKI, however, especially for the lung cancers that are with activating EGFR mutation but negative for T790M. Of note, most studies focused on single gene alteration, but it is needed to be indicated that multiple resistance mechanisms may co-exist because of tumor heterogeneity (9,10). Therefore, novel perspective study for EGFR-TKI resistance is needed.

Genomic instability involves a transient phase of tetraploidization. Tetraploid cells can undergo asymmetric cell division or chromosome loss, leading to increased tumor heterogeneity and multidrug resistance (11). Experimental evidence further revealed that chromosomal instability enables tumor adaptation with aneuploid fitness landscape (12,13). In lung cancer cells, chromosome 7 aneuploidy was found to be one of the most important events for cancer development. These deregulations or variations of Chromosome 7 can be frequently detected in malignant lung cancer and pre-cancerous lesions cells, or even in lung bronchial cells, but not in health lung tissues cells (14-16). Chromosome aneuploidy detection has been used for prenatal tests through plasma cell free DNA, with minimal false positives and false negatives (17). Similar to fetal tissues and cells, tumors also keep shedding DNA into peripheral blood stream. As such, technology of ctDNA (circulated tumor DNA) has been successfully applied in clinic to detect biomarkers or cancer somatic mutations such as EGFR mutations for predicting potential benefits of targeted therapies. In addition, detection of chromosomal copy number changes with ctDNA technology has also been reported in patients with breast cancer (18), hepatocellular carcinoma (19) and lung cancer (20).

The criteria for clinical defining intrinsic EGFR-TKI resistance for lung cancer have not been established. Jackman *et al.* (21) proposed criteria for acquired resistance of EGFR-TKI in lung cancer patients with mutant EGFR, including that patients achieve a partial or complete response or develop a stable disease in response to EGFR-TKI monotherapy (>6 months). Of note, patients with PFS less than 6 months were recruited in the study, and thus the lung cancer of these patients potentially harbored intrinsic resistant to EGFR-TKI treatment.

Low pass whole genome sequencing approach with an optimized bioinformatics pipeline, ultra-sensitive chromosomal aneuploidy detector (UCAD), were used to screen chromosomal aneuploidy, especially chr7 aneuploidy, by using plasma cell free DNA in EGFR targeted therapy intrinsic resistant patients.

Methods

Patients

Thirty-one lung cancer patients and ten health volunteers were enrolled in this study. All lung cancer patients relapsed after EGFR-TKI treatment. The protocol of the study was approved by the Institutional Review Board of Hangzhou First People's Hospital (No. HZFH CA15-02). All recruited patients and health volunteers have signed a written

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informed consent.

Sample collection and DNA extraction

Blood samples were collected within 14 days after the development of TKI resistance as assessed by the physician according to the Jackman criteria (21) and before the start of the following treatment. Approximately 10–15 mL of peripheral blood was collected in a cell-free DNA protection vacuum tube (AmoyDx, Xiamen, Fujian, China), which contains a cell-free DNA protection reagent to keep DNA stable for 7 days at 4–25 ℃. Blood samples were transported to the Center for Translational Medicine of Hangzhou First People's Hospital within 36 hours for further processing. For DNA extraction, the blood samples were centrifuged at 2,500 ×g for 10 minutes at 4 ℃, and the supernatant was transferred to a new tube for further centrifugation at 15,800 ×g for 15 minutes at 4 ℃. The collected plasma supernatant was then stored at −80 ℃. Cell-free DNA from 1.5 mL plasma was extracted with a QIAamp Circulating Nucleic Acid kit according to the manufacturer's instructions (Qiagen, Hilden, Germany).

ARMS assays for testing EGFR T790M mutations

The EGFR T790M mutational status was determined by ARMS (amplification refractory mutation system) with the ADx-ARMS kit (AmoyDx, Xiamen, China). *EGFR* mutations in plasma ctDNA were detected by using the plasma EGFR detection kit on qPCR platform. All experiments and genotype calling were performed according to the manufacturer's instructions (22).

Next generation sequencing

Next generation sequencing was performed as previously described (19,23). Briefly, DNA was fragmented into an average size of 300 bp (cfDNA without fragmentation), and 100 ng of fragmented genomic DNA (or 10 ng for cfDNA) was used for preparation of sequencing libraries (NEBnext Ultra II). Eight bp barcoded sequencing adaptors were then ligated to the DNA fragments and the DNA templates were amplified by PCR. Purified sequencing libraries were massively parallel sequenced by Illumina HiSeq Xten platform. 4G sequencing raw data per sample were filtered and aligned to the human reference genome.

Gene-level copy number analyses

Chromosome copy number aberrations (CNAs) were determined with the Ultrasensitive Chromosomal Aneuploidy Detector (UCAD) pipeline. Sequencing coverage for each 200 K bin was calculated followed by GC normalization. The sequencing coverage were further normalized by controls samples. The Z-score for each bin was calculated by formula,

$$Z = \frac{C_{test} - mean(C_{control})}{stdev(C_{control})} \quad [1]$$

where C_{test} and $C_{control}$ are the coverage of the bin. The normalized bin values were sent to segmentation calls by algorithm circular segmentation algorithm as provided by R package DNAcopy. Samples with standard deviation of copy ratios between the adjacent bins >30 for genome-wide results were considered as with poor-quality sequence data, and these samples were excluded from this study.

Statistical analysis

R package 'DNACopy' was used for analysis of copy number changes. A P value of <0.05 was considered as statistically significant binary segmentation. Absolute segment value is used for further analysis. The sensitivity and specificity of UCAD were estimated by ROC curves. The chi-square test was used for categorical variables. OS was calculated from the time of development of TKI resistance to the time of death of any reason or last follow-up. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. All statistical analyses were performed using SPSS 17.0.

Results

Patient characterization

In this study, thirty-one enrolled lung cancer patients were collected from a previous study (clinical trial NCT02418234) which aimed to analyze the association of clinical mode and plasma T790M mutation in the relapsed patients after treatment with first generation TKI (24). Of them, 18 patients were with PFS less than 6 months and thus the tumors were considered to be with primary resistance to EGFR-TKI, 13 patients were with PFS longer than 36 months and were defined with

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Table 1 Clinicopathological information

Clinical factors	Intrinsic resistance, N=18	Acquired resistance, N=13
Gender		
Male	9	6
Female	9	7
EGFR baseline		
L858R	7	4
19del	9	9
19del+L858R	1	0
G719X	1	0
Drug types		
Gefitinib	9	9
Icotinib	9	1
Erlotinib	0	3
Age (years)	57	67
≤60	11	5
>60	7	8

acquired EGFR-TKI resistances. As shown in *Table 1*, plasma T790M mutation is negatively detected in all patients by using Amplification Refractory Mutation System PCR. No statistical differences were observed for gender and EGFR baseline mutations between the two groups with primary and acquired EGFR-TKI resistance. Of note, clinical trial NCT02418234 involved 307 patients, and only these thirty-one plasma T790M negative patients shown here were found the PFS less than 6 months or more than 36 months, and our data showed that patients with primary resistance tend to be 10-year younger than that with acquired resistance. With 60-year as a cutoff value, we did not find statistical significance for the incidence of patients between these two groups.

Cell-free DNA whole genome copy number profiling

In this study, all raw sequencing reads were mapped to human reference genome hg19, and genomic coverage was counted by using software samtools mpileup. With these setting, we counted the average coverage for each 200k bin, and determined the significant genomic breakpoints with using circular binary segmentation algorithm. Our results showed that in all 31-tumor specimen, when compared to normal control, chromosomal breakpoints were commonly detected on centromere regions and chromosomal arm coverage imbalances were found on chromosome 1, 7 and 8 by visual inspections. In addition, chromosome 1 short arm (1p) was found with coverage lower than normalized average (as calculated as 0), indicating 1p copy loss, and chromosome 1 long arm (1q) was found with coverage higher than the normalized average, indicating 1q copy number gains. Similarly, chromosome 7 short-arm was found with higher coverage comparing to long arm, indicating chromosomal arm copy gains. Analysis further revealed a statistically significant focal amplification on chromosome 7p11.2, a loci where EGFR located. We also noted a copy number gain peak around 7q31.2. Of interest, oncogene MET, a well-known cancer driver, is located in this loci (*Figure 1*). In these analyses, the statistical significance of copy gain/loss was calculated by Student *t*-test by comparing chr1p 200-bin coverage values against that of health controls.

Comprehensive analysis further demonstrated statistically significant chromosome arm level changes between tumors with intrinsic resistance and with acquired resistance. *Figure 2* shows a heatmap illustrating the most significant genomic changes detected. These include genetic events of 7p gains (Z ≥3 in 8/18, 44.4%), 1q heavy gains (Z ≥6 in 5/18, 27.8%), 7q31.2 gains (Z ≥3 in 2/18, 11.1%) and 7p11.2 heavy gains (Z ≥6 in 5/18, 27.8%), frequent chromosome losses of chr18 (7/18, 38.9%), chr9 (6/18, 33.3%), chr3 (6/18, 33.3%), 16p (1/18, 5.56%), 1p (7/18, 38.9%), chr20 (10/18, 55.6%), 22q (4/18, 22.2%), 10q (8/18, 44.4%), 8p (3/18, 16.7%) and chr15 (6/18, 33.3%) and chromosomal gains of 7q (5/18, 27.8%), 7p (8/18, 44.4%), 8q (4/18, 22.2%) and 1q (6/18, 33.3%) that are exclusively found in the group with intrinsic resistance of EGFR-TKI. On the other hand, genetic events with copy loss in chr18 (4/13, 30.8%), chr9 (1/13, 7.69%), chr3 (4/13, 30.8%), 16p (2/13, 15.4%), 1p (3/13, 23.1%), chr20 (6/13, 46.1%), 22q (1/13, 7.69%), 10q (6/13, 46.1%), 8p (1/13, 7.69%) and chr15 (2/13, 15.4%), and genetic events with copy gain in 7q (3/13, 23.1%), 8q (1/13, 7.69%) and 1q (1/13, 7.69%) were detected in the specimen from patient with lung cancer that are T790M negative but with acquired resistance to EGFR-TKI.

In addition, non-arm level copy number gains in 7p11.2, 22q11.2, 16p11.2 and 5q31.3, including focal amplifications, were detected in specimen from patients with lung cancer that were defined as with intrinsic resistance of EGFR-TKI with frequencies of 66.7% (12/18), 22.2% (4/18), 33.3%

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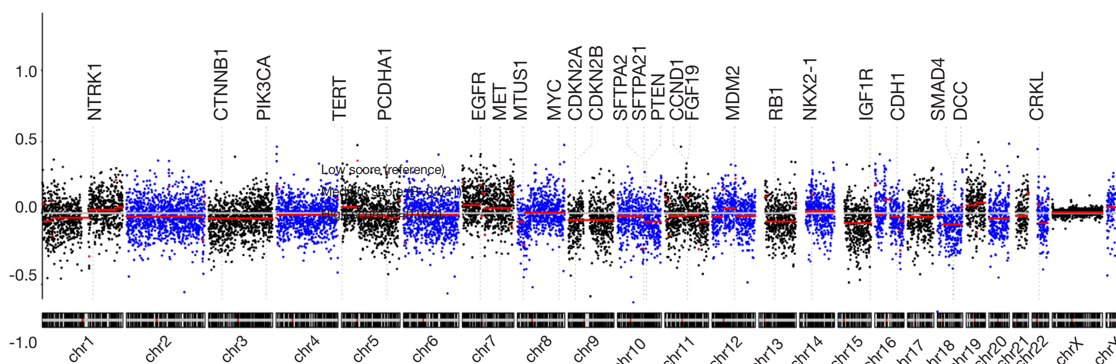

Figure 1 Plasma cell free DNA somatic copy numbers of relapsed lung cancer patients. Chromosome 1 to 22 is layout from left to right with green and black colors. Chromosomal segments are marked in red lines. Representative genes for the segments of interest are marked on the top.

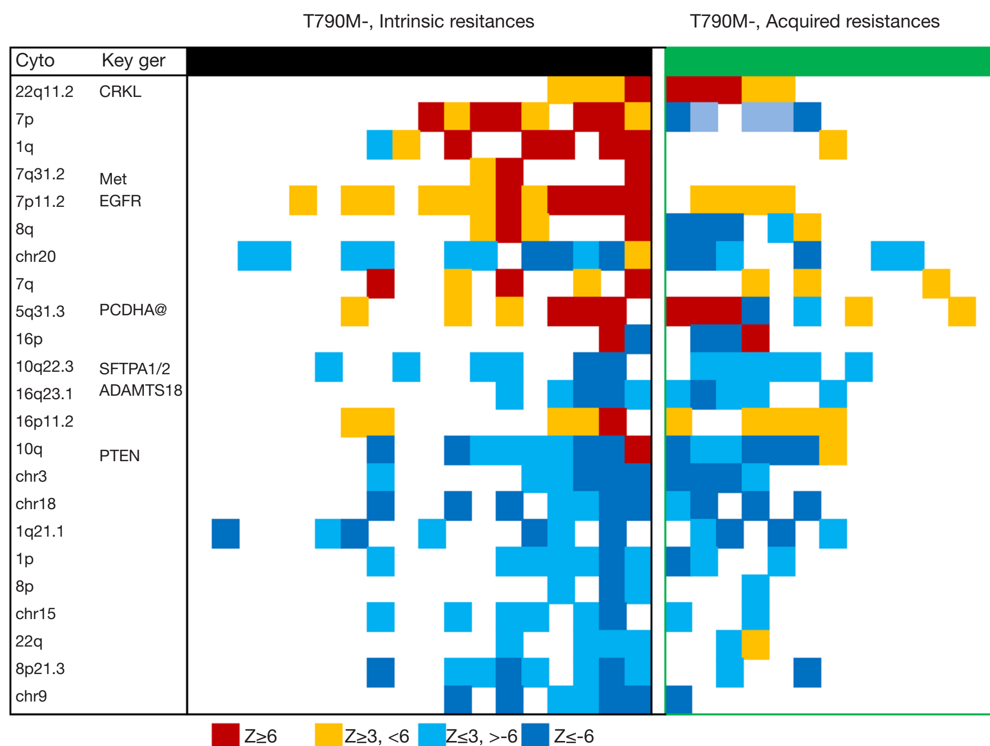

Figure 2 Heatmap view of chromosomal changes between intrinsic and acquire patients. Heatmap view of segment copy number changes in T790M negative intrinsic resistance and acquired resistance, with each line represent a segment and each column represent a sample. Significant copy-gain segments with Z-score larger than 6 are marked in red color, Z-score between 3 and 6 in orange. Significant copy-loss segments with Z-score less than -6 are marked in dark blue, Z-score between −3 and −6 in light blue. The rest data points with Z-score between −3 and 3 are in white.

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Table 2 Statistical analyses of somatic copy number changes in drug resistance groups

CNV	T790M-		P value
	Intrinsic resistant, N=18	Acquired resistant, N=13	
7p gain	8	0	0.009
7p11.2 (EGFR) gain	12	4	0.073
7q31.2 (MET) gain	3	0	0.245
1q gain	6	1	0.191

All the other events in *Figure 2* were not listed here because of no significance.

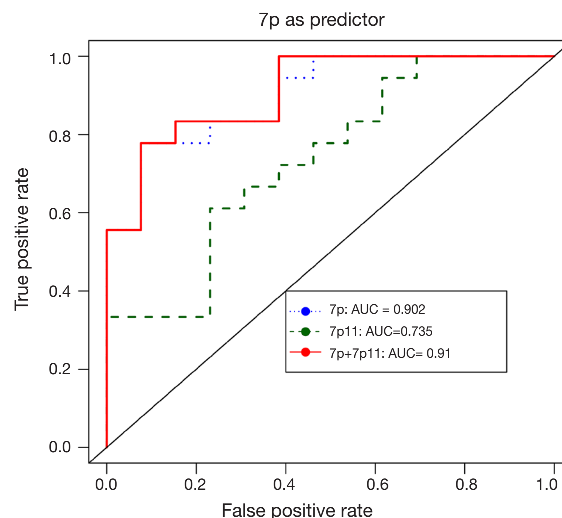

Figure 3 EGFR copy number as a predictor for EGFR-TKI intrinsic resistance in T790M negative patients. AUC curves for 7p gains to predict intrinsic resistance. The performance of 7p11 is indicated in green dash line. The performance of 7p is indicated in blue dot line. The performance of combining 7p and 7p11 is indicated in red line. The combining product was estimated by 10.7×7p+0.619×7p11. The coefficients were estimated by logistical regression Category~logit (7p+7p11), where Category is the category label of a sample, which is either 'intrinsic resistance' or 'acquired resistance'.

(6/18) and 33.3% (6/18), and in patients with lung cancer that were with acquired resistance with frequencies of 30.8% (4/13), 38.5% (5/13), 53.8% (7/13) and 38.5% (5/13), respectively (*Figure 2*). Of interest, all these amplified locations are linked with potential lung cancer oncogenes. For example, lung cancer oncogene EGFR locates in 7p11.2 (1); CRKL, a recently identified lung cancer driver predicting the relapse of patient after TKI treatment (25), locates in 22q11.2; ADAMTS18 which contributes to lung cancer development (26) locates in 16p11.2. PCDHA, methylation of this gene has been reported to be involved in multiple caner development (27), locates in 5q31.3.

chr7p gains is a frequent event detected in patients with lung cancer that are EGFR T790M negative but with intrinsic TKI resistance

We next examined the potential correlations of chromosomal copy number changes with TKI resistances. Of interest, our data showed that chromosome 7 copy number gain significantly correlates with intrinsic TKI resistances: 7p11.2 (EGFR loci) gains was detected in 66.7% (12/18) of lung cancer patients with intrinsic TKI resistance while it is found in only 30.7% (4/13) of patients with acquired TKI resistance; 7p arm gains were found in 44.4% (8/18) of patients with intrinsic resistance, and no such event was detected in patients with acquired TKI resistance (Fisher exact test, P=0.009). However, no statistical significance was found for the other frequent detected genomic events as listed in *Table 2*, including 8q gain, 8p21.3 loss and 1q gain. The operating characteristic (ROC) analysis further revealed that chr7p copy number gains of chr7p can serve as a predictor for distinguishing intrinsic resistance from acquired resistance for lung cancer in response to EGFR-TKI (*Figure 3*).

Copy number changes correlate with overall survival (OS) after TKI resistances

We also determined the potential correlations between these observed chromosome changes and OS of patient (*Figure 4*). For this, patients were grouped with short OS (≤6 months), medium OS (6–12 months) and long OS (≥12 months). We found that chr9 loss and 1q gain significantly correlated with shorter OS (trend tests, P=0.020 and 0.029 respectively). In particular, patients with 1q gains detected had shorter OS (medium average of OS for 3.4 months) when compared to patients with 1q silent of lung cancer (medium average of OS for 22.2 months, hazard ratio =10.97, log rank test P=0.029). The similar association of chromosomal changes with poor OS were also observed with chr9 loss (hazard ratio =6.48, P=0.020), and 7q31.2 gain (hazard ratio =1.37, P=0.031) where oncogenes

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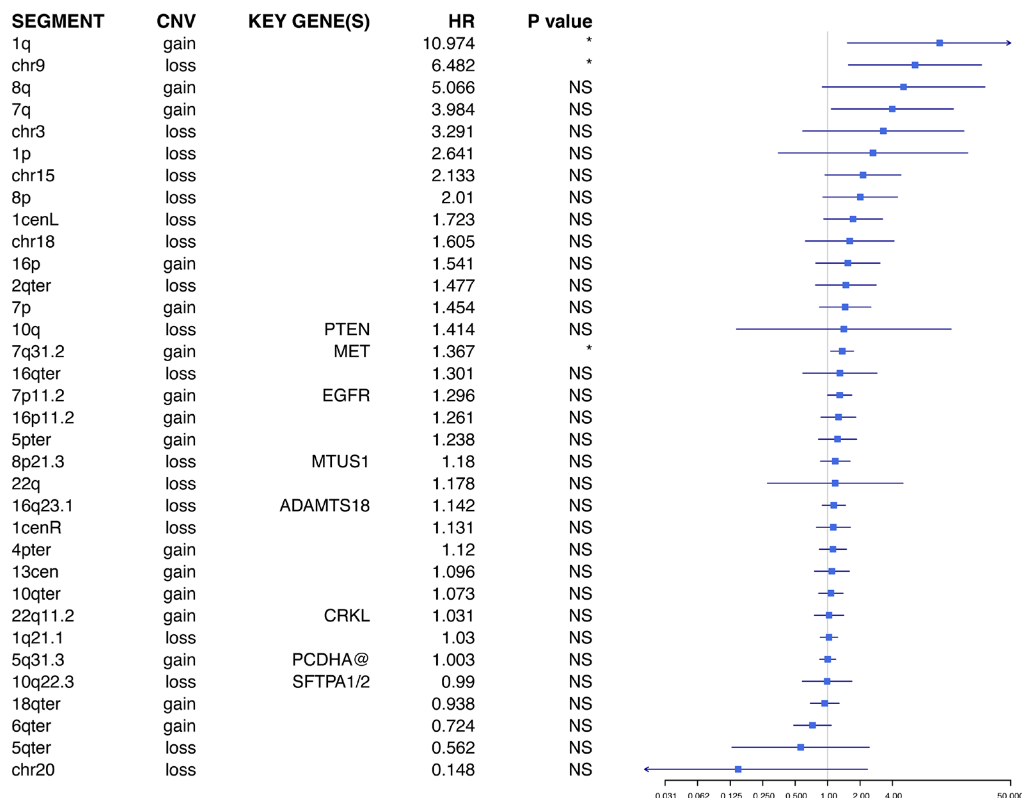

Figure 4 Genetical events which predicts overall survival. Forest plots for odd ratios of predicting intrinsic resistances for each chromosomal segment. The odd ratios are estimated by logistical regression Category~logit (segment_of_interest), where Category is the category label of a sample, which is either 'intrinsic resistance' or 'acquired resistance'. Odd ratios are sorted and plot from top to bottom. Statistically significant segments (P value <0.05) are marked with stars (*).

CDKN2A/B and MET locate (28,29). No such correlations were found for other genetic events such as 8p loss, 1p loss, 8p21.3 loss, 7q gain, 8q gain and 16p11.2 loss, however.

Discussion

EGFR-targeted therapy has shown superior efficacy for patients with EGFR mutation. However, about 20% to 30% patients with advanced stage lung cancers that have EGFR activating mutation show intrinsic resistance to TKI. Understanding the mechanism of the intrinsic EGFR-TKI resistance is thus important for helping improve clinical practice for lung cancer.

In this study, we identified multiple somatic copy number variations (CNV) of chromosome through plasma cfDNA sequencing. These variants include chromosome arm level changes, focal amplifications and deletions. In mammalian cells, chromosome mis-segregation can result in chromosomal arm level changes, which affects the gene structure of many tumor genes, such as PTEN deletions on chr10 and SMAD4 deletions on chr18 (30,31). Focal amplifications, or focal chromosomal CNAs, have been discovered in cancer as critical genetic events of cancer driver gene activation resulting from many selection events during the evolution of cancer genomes (32). Our results showed that chromosomal breakpoints on centromere regions and CNV of chromosomes on Chromosome 1 (1p copy loss and 1q copy gain) and chromosome 7 (7p11.2 focal amplification and 7q31.2 copy gain) are frequently detected in plasma cfDNA of patients that were resistant to EGFR-TKI (as with either intrinsic or acquired resistance). Other chromosomal genetic variations detected frequently in the plasma cfDNA of these patients include chromosome losses of chr19, chr9, chr3, chr20, chr15, 16p, 22q, 10q and 8p, and chromosomal gains of 8q. Of interest, data analysis further revealed the certain patterns of chromosomal

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somatic CNV that may correlate to the intrinsic or acquired resistance to EGFR-TKI treatment in lung cancer patients. For example, 7p gains detected in the plasma cfDNA achieve highest correlation with predictable intrinsic EGFR-TKI resistance (ROC =0.93).

As regarding these chromosomal genetic variations, it is of interest that many lung cancer driver genes or tumor suppressor genes locate in the chromosome locus that have detectable changes in patients with lung cancer that are resistant to EGFR-TKI, as shown in this study. Of them, PCDHA gene cluster are the most commonly hyper-methylated genes discovered in human cancers (26,27). CRKL and EGFR are two well-studied lung cancer oncogenes: CRKL amplification was discovered as one of the acquired resistances to kinase inhibitors in lung cancers treated with EGFR inhibitors (25); EGFR amplification is one of the most common genetic events in lung cancer (33), and gain of EGFR amplifications has been proposed as one of the potential drug resistance mechanism of EGFR tyrosine kinase inhibitors (34,35). In our research, EGFR (7p11.2) copy gains were detected in 16/31 (51.6%) plasma samples. CRKL (22q11.2) copy gains were detected in 8/31 (25.8%) plasma samples. Of note, however, CRKL and EGFR copy gains were detected in separate samples (*Table S1*), it is thus suggested that these two genetic events, if they are corresponding to, are independent to EGFR-TKI resistance. On the other hand, our study also suggest that EGFR gene amplification can drive EGFR-TKI resistance, with gene amplification may results in a ligand independent kinase domain activation leading to intrinsic TKI resistance while secondary EGFR mutation is a result of genetic selection for gained resistance during TKI treatment.

TKI resistance remains as a clinical challenge for lung cancer management. In this study, we determined the chromosomal somatic copy number changes in plasma samples of patients with TKI resistance by using clinical achievable pipeline ultra-sensitive chromosomal aneuploid detector (UCAD). Our results not only identified the most frequently detectable chromosomal somatic copy number variants that are associated with TKI resistance, but also revealed patterns that may specifically correlate with either intrinsic or acquired EGFR-TKI resistance. Our data also demonstrated that some of the chromosomal somatic copy number variants such as 7p gain and 1q gain predict worse survival of the patients. These novel findings have significant clinic impacts for guiding lung cancer treatment. For examples, EGFR gene amplification (7p gain) in lung cancer cells may result in intrinsic resistance to TKI, and these patients may benefit from clinical management with addition of cetuximab, an anti-EGFR monoclonal antibody that target EGFR amplification; Chr1q gain is another frequent genetic event identified in lung cancer patients with intrinsic resistance to TKI, and 1q21.3-encoded S100 calcium-binding protein (S100A) family members and IL-1 receptor–associated kinase 1 (IRAK1) which can be targeted by a small-molecule kinase inhibitor, pacritinib; The examination of the potential 7p gains and 1q gain in the plasma samples of lung cancer patients with UCAD may provide a useful tool for monitoring EGFR-TKI response and drug resistance assessment in patient.

Conclusions

In this study, we identified multiple somatic CNV in distinguishing EGFR-TKI intrinsic and acquired resistance through plasma cfDNA sequencing. The results were from a small-scale prospective study, including 31 cancer patients and 10 health controls. The data present here uncovered encouraging findings for mechanism and biomarkers for EGFR-TKI resistance. However, a large prospective clinical trial to further confirm these discoveries is urgently needed.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The protocol was approved by the Institutional Review Board of Hangzhou First People's Hospital (No. HZFH2015-47-01). All patients signed the informed consent.

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Table S1 CRKL (22q11.2) copy gains, EGFR (7p11.2) copy gains and 7p gains in T790M primary and acquired resistance samples

Molecular group	Patient group	ID	Z–scores		
			22q11.2	7p	7p11.2
	T790M– AR	AD15085	8.13	–6.27	1.41
22q11.2 positive, 7p positive	T790M– AR	AD15077	7.74	–5.81	4.71
	T790M– AR	ST016	3.92	–5.26	3.30
	T790M– AR	AD15054	3.08	–3.08	5.10
	T790M– AR	AD15055	8.27	1.94	5.00
	T790M– PR	ST118	7.35	4.13	10.89
	T790M– PR	ST017	4.55	6.57	6.32
	T790M– PR	AD15132	3.49	6.02	7.79
	T790M– PR	AD15124	4.29	1.70	7.90
	T790M– PR	AD15062	0.10	41.39	17.30
22q11.2 negative, 7p positive	T790M– PR	AD15145	–0.89	2.93	5.92
	T790M– PR	ST074	0.51	5.91	4.19
	T790M– PR	AD15065	2.15	2.33	3.86
	T790M– PR	AD15162	0.99	1.61	3.60
	T790M– PR	ST041	0.46	–0.64	3.44
	T790M– PR	E15073B	0.10	4.36	3.40
	T790M– PR	AD15063	2.25	6.92	3.20
22q11.2 negative, 7p negative	T790M– PR	AD15029	1.19	2.59	1.46
	T790M– PR	E15114B	1.53	1.61	0.63
	T790M– PR	AD15076	1.39	1.56	1.12
	T790M– PR	AD15138	0.91	1.34	2.35
	T790M– PR	ST004	0.48	0.05	0.81
	T790M– PR	AD15163	0.38	–0.29	1.12
	T790M– AR	ST100	0.48	1.45	1.50
	T790M– AR	AD15146	0.26	1.41	–0.57
	T790M– AR	ST121	0.16	0.71	2.81
	T790M– AR	AD15060	1.52	0.46	0.38
	T790M– AR	ST040	0.95	–0.34	1.12
	T790M– AR	ST038	–0.14	–1.13	0.78
	T790M– AR	AD15091	–1.40	–1.49	–0.02
	T790M– AR	AD15061	–0.82	–8.83	–1.28
	HEALTH_CTRL	PG10	1.17	1.42	0.66
	HEALTH_CTRL	PG09	0.54	1.13	0.06
	HEALTH_CTRL	PG07	0.36	0.71	–1.21
	HEALTH_CTRL	PG13	0.27	0.60	1.77
	HEALTH_CTRL	PG04_L3	–1.97	0.51	–0.69
	HEALTH_CTRL	PG11	0.37	0.48	1.00
	HEALTH_CTRL	PG15	–0.01	0.22	–1.30
	HEALTH_CTRL	PG08	0.18	–0.44	–1.15
	HEALTH_CTRL	PG12	0.50	–0.66	0.07
	HEALTH_CTRL	PG06_L2	1.10	–0.84	1.12
	HEALTH_CTRL	PG14	–0.76	–1.31	–0.45
	HEALTH_CTRL	PG05_L3	–1.75	–1.81	0.13

AR, acquired resistance; PR, primary resistance.