Experimed 2024; 14(1): 26-39

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1408284

Cytogenetic and FISH Examination of 3p Abnormalities in Lung Cancer Patients*

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Cite this article as: Bakhshaliyeva N, Cirakoglu A, Ongen HG, Bil Tuncay H, Tarkan Arguden Y. Cytogenetic and FISH examination of 3p abnormalities in lung cancer patients. Experimed 2024; 14(1): 26-39.

ABSTRACT

Objective: Deletions or loss of heterozygosity in chromosome 3p are very common in small-cell lung cancer (SCLC) and lung adenocarcinoma (ADC) cases. These are typically found in tumor cells but rarely observed in lymphocytes. This study aimed to evaluate the frequency of 3p deletions and/or abnormalities in the blood of lung cancer patients using conventional cytogenetics and fluorescence *in situ* hybridization (FISH), by targeting the fragile histidine triad diadenosine triphosphatase (FHIT) gene located at the commonly deleted region of 3p14.2, in lung cancers.

Materials and Methods: The study examined 24 SCLC patients, 30 ADC patients, and 20 healthy controls. It used standard procedures to perform a 72-h lymphocyte culture, G-banding, and FISH.

Results: All patient group cases showed multiple numerical and structural abnormalities, with numerical abnormalities being more prominent and involving all chromosomes. The following two 3p abnormalities were detected in one patient: del(3)(p22) and t(3;5)(p25;q31). FISH showed positive results regarding FHIT deletion in 9 (30%) ADC, and 7 (29%) SCLC patients.

Conclusion: Regardless of the rarity of 3p abnormalities in lymphocytes, a high frequency of chromosomal aberrations may indicate genomic instability. Nevertheless, due to being a time-consuming and expertise-requiring technique, conventional cytogenetics is not recommended for lung cancer monitoring. However, the FISH results suggested that using FISH to examine FHIT gene status in lymphocytes could be a promising biomarker for lung cancer.

Keywords: Small cell lung carcinoma, FISH technique, adenocarcinoma, cytogenetics, chromosome 3, FHIT

INTRODUCTION

Lung cancers are one of the main types of cancer-caused deaths, worldwide (1). Many studies have shown the effects of genetic factors on lung cancer. Small cell lung cancer (SCLC) covers 20-25% of all lung cancers; it has a different clinicopathological course involving paraneoplastic syndromes and a tendency to metastasize, requires an aggressive clinical process, and is insensitive to chemotherapy and radiation (1, 2). Lung adenocarcinomas (ADC) comprise nearly 40% of all lung cancers (3). 3p abnormalities are the most common chromosomal abnormalities in SCLCs and ADC, and cytogenetic studies have shown 3p deletion to be a characteristic finding for small cell and ADC lung cancers (4-9). Even when no chromosome 3p anomalies are

*This study was produced from the master's thesis and was presented at 13th Balkan Congress of Human Genetics and 13th National Congress of Medical Genetics with International Participation.

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Submitted: 23.12.2023 **Revision Requested:** 18.01.2024 **Last Revision Received:** 31.01.2024 **Accepted:** 13.02.2024 **Published Online:** 25.03.2024

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found, a loss of heterozygosity in 3p is shown (5, 8, 10). With cytogenetic and molecular genetic techniques, 3p deletion or 3p loss of heterozygosity has been observed in 100% of SCLC cases and 80% of ADC cases (4, 8-13). These abnormalities are typically detected in a patient's tumor cells, but rare cases exist where this has been observed in lymphocyte cultures from peripheral blood samples (11, 14, 15). Although these studies have shown chromosomal abnormalities specific to SCLC to be able to be seen in blood samples, the number of cases is insufficient to understand whether these findings can be used to obtain information about the course of the disease. Because peripheral blood is easy to collect and manipulate and suitable for recurring examinations, knowing whether cancer-specific chromosomal abnormalities in peripheral blood are common enough to be used as indicators are desirable for the prediction of cancer development, prognosis, and metastasis.

Similar to most epithelial tumors, lung cancer consists of the accumulation of multiple genetic and/or epigenetic changes that can result from deletions, mutations, or changes in gene methylation. Chromosomal deletions are associated with regions of tumor suppressor genes (16). Recent studies have identified various tumor suppressor genes (TSGs) that play or may be able to play a role in carcinogenesis in regions of chromosome 3 that are also deleted in SCLC and ADC cases (4, 5, 7, 9, 17). For example, many TSGs such as fragile histidine triad diadenosine triphosphatase (FHIT) at 3p14; ROBO/DUTTI at 3p12; and Ras association domain family member 1 alpha (RASSF1a), the histocompatibility allele H-37, FUS RNA-binding protein (FUS1), and semaphorin 3B (SEMA3B) at 3p21 are lost as a result of these deletions (5, 7, 9, 16, 18). Other TSGs found in chromosomal regions commonly deleted in lung cancers include the adenomatous polyposis coli (APC) regulator of WNT signaling pathway (5q21), retinoblastoma (RB) (13q), p53 (17p), and p16 (9p21) (11). The FHIT gene is involved in the accumulation of di-adenosine tetraphosphate, thus causing DNA synthesis and proliferation. It is located at 3p14.2 and overlaps with a common fragile site known as Fra3B, which is prone to damage and leads to chromosome aberrations. Loss or reduced expression of FHIT is found in preneoplastic lesions and cancers, including lung cancer. This results in replication stress, DNA breaks, aneuploidy, copy-number changes small insertions and deletions, and point mutations (19).

This study aimed to use conventional cytogenetics and fluorescence *in situ* hybridization (FISH) techniques to evaluate the frequency of 3p deletions and abnormalities in the peripheral blood samples of lung cancer patients.

Based on the information in the literature, the study has deemed the inclusion of lung ADC to be appropriate, considering its similarity to SCLC in terms of such features as carrying 3p anomalies and its aggressive course. The study used a FISH probe for the FHIT gene located on 3p14.2, which is within the commonly deleted region of 3p in lung cancers, in order to be able to detect deletions smaller than the scope of what cytogenetics can detect. The FHIT gene has been chosen from among the numerous genes residing in the relevant

Table 1. Clinical characteristics of the cases

	ADC (n = 30)	SCLC (n = 24)	Control (n = 20)
Gender			
Female	10	4	12
Male	20	20	8
Age (year), min-max (median)	46-87 (61.5)	47-90 (57)	31-51 (44)
Grade			
1	4	-	
2	8	3	
3	3	13	
4	8	8	
Metastase	13	8	
Bone	4	1	
Brain	3	4	
Liver	2	-	
Esophagus	2	-	-
Head-neck	-	1	
Brain+Bone	1	-	
Liver+Bone	1	1	
Liver+Muscle	-	1	
Smoking	14	5	-

ADC-adenocarcinoma; SCLC-small-cell lung cancer

region because it is a known tumor suppressor gene and has already been associated with lung cancer (5, 7, 18, 20-22). The study used conventional cytogenetic techniques to examine chromosomal abnormalities in peripheral blood cultures and used the FISH method to investigate the loss of the FHIT gene in the 3p14 region.

MATERIALS AND METHODS

Subjects

The study has enrolled a total of 54 untreated lung cancer cases (24 SCLC and 30 ADC) and 20 healthy (non-smoker≥30 years of age) control subjects. The study has been conducted in accordance with the Helsinki Declaration and was approved by the Cerrahpasa Faculty of Medicine Medical Ethics Committee (Reference No. 83045809-604.01.02-A49), with all patients and individuals in the control group having signed informed consent forms. Of the 30 ADC cases, 10 were female, and 20 were male. Of the 24 SCLC cases, four were female, and 20 were male. Of the 30 ADC patients, 16 were smokers, and 14 were non-smokers. Of the 24 SCLC patients, 19 were smokers, and 5 were non-smokers. The median age was 61 for the ADC cases and 57 for the SCLC cases, while the median age was 63 for ADC and 60 for SCLC. Metastases were detected in 13 of the 30 ADC patients and in 8 of the 24 SCLC patients. Table 1 lists the clinical characteristics of the cases. The study used conventional cytogenetics to examine 3p and other chromosomal abnormalities in the peripheral blood samples of all patients and control subjects and used FISH techniques to examine their FHIT gene status.

Conventional Cytogenetics

A standard 72 h lymphocyte culture procedure was applied to the heparinized blood samples of the patients and control subjects. Giemsa-Trypsin-Leishman (GTL) banding was used for chromosome banding, and metaphases were evaluated in accordance with the International System for Human Cytogenomic Nomenclature (ISCN) 2016. Consistent with the

Table 2. Distribution of structural anomalies between SCLC and ADC disease

Abnormality	Case Number		Metastatic case
	SCLC	ADC	
del(18)(p11)	2	4	A16-Esophagus Met K9-Brain Met
del(6)(q15q21)	1	3	A2-Bone Met
del(22)(q12)	1	1	A2-Bone Met
del(X)(p11)	-	1	-
del(3)(p22)	1	-	-
t(3;5)(p25;q31)	1	-	-
add(3)(q29)	-	1	-
del(7)(p13)	-	1	A16-Esophagus Met
i(7)(p10)	-	1	A30-Brain Met
i(7)(q10)	-	1	A30-Brain Met
inv(9)(p12q13)	1	-	-
dic(9;11)(q34;p15)	-	1	A2-Bone Met
inv(10)(q23q24)	-	1	A12-Bone Met
add(10)(q26)	-	1	A2-Bone Met
del(14)(q24)	-	1	A12-Bone Met
chtg(16)(q21)	-	1	-
der(18)del(18)(p11)del(18)(q12q22)	1	-	-
del(20)(q13)	1	-	K17-Brain Met

ADC-adenocarcinoma; SCLC-small-cell lung cancer; Met: Metastasis

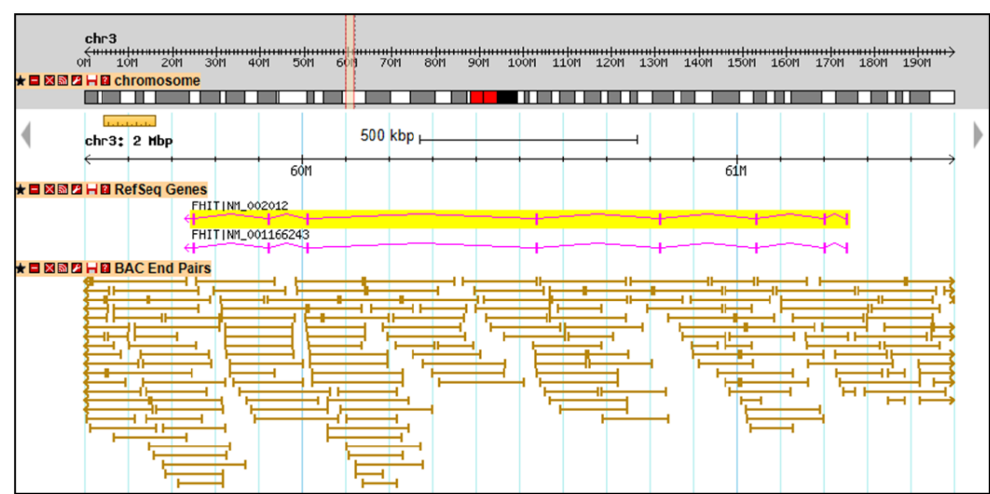

Figure 1. The 3p14.2 (FHIT) DNA FISH probe.

ISCN rules, the chromosomal gains and structural abnormalities observed in two metaphases and chromosome losses observed in three metaphases were considered clonal. Whenever possible, 100 metaphases were evaluated for each case, of which 20 were captured and analyzed on an image analyzer (Ankagen/IMGESS/Karyotyping Gv2.5) and 30 were analyzed under the microscope. If no chromosomal abnormality was present in these metaphases, another 50 metaphases would then be scored for chromosome 3 abnormalities. If 3p or any other chromosomal abnormality was observed in any stage, all metaphases of the case were captured and analyzed on the image analyzer.

FISH

FISH analyses were performed using a custom-designed, quality-controlled FHIT probe (provided by Medimiks Medical Biotechnology Systems & Services Ltd.) The centromere probe for chromosome 3 was used for the control signal (Figure 1).

Table 3. Statistical results of patients with FHIT anomaly diagnosed with ADC and SCLC according to clinical status variables

	SCLC (24)	ADC (30)	p-value
Age (year), median (min-max)	59.83 (47-90)	63.07 (46-87)	0.166
FHIT, n (%)			
Deletion positive	7 (29.2)	9 (30)	0.947
Deletion negative	17 (70.8)	21 (70)	
Metastasis, n (%)			
Yes	8 (33.3)	13 (43.3)	0.454
No	16 (66.7)	17 (56.7)	
Grade, n (%)			
Grade I	0 (0)	4 (13.3)	**0.015**
Grade II	3 (12.5)	8 (26.7)	
Grade III	13 (54.2)	5 (16.7)	
Grade IV	8 (33.3)	13 (43.3)	
Smoking, n (%)			
Yes	19 (79.2)	16 (53.3)	**0.048**
No	5 (20.8)	14 (46.7)	
Gender, n (%)			
Female	4 (16.7)	10 (33.3)	0.165
Male	20 (83.3)	20 (66.7)	

***ADC-adenocarcinoma; SCLC-small-cell lung cancer**

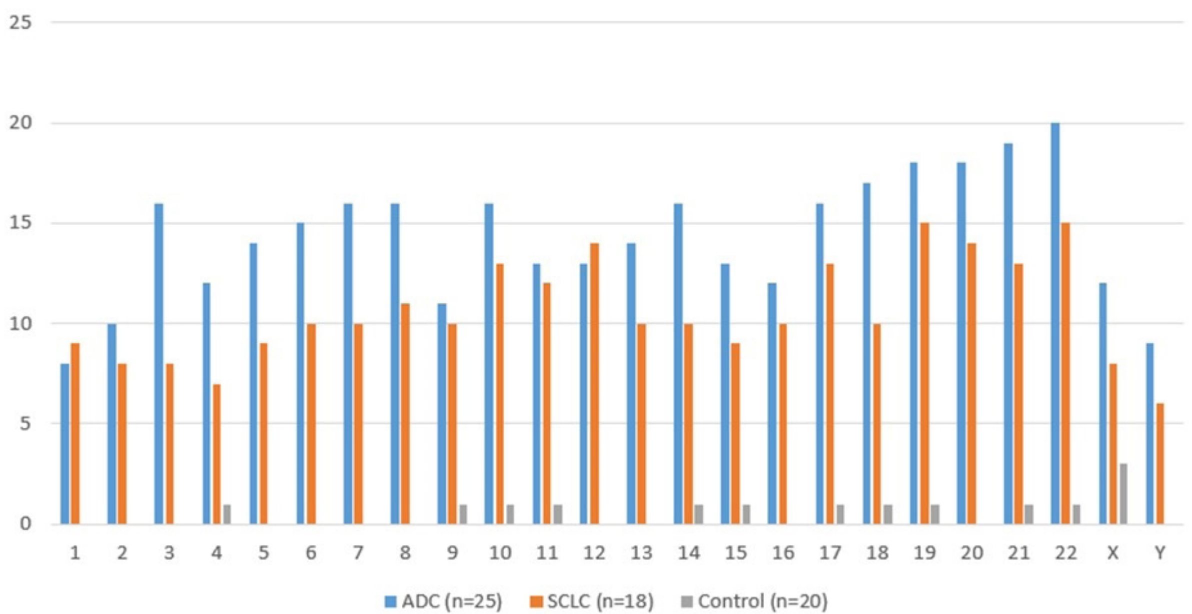

Figure 2. The distribution of monosomies over all the chromosomes between the SCLC and ADC cases.

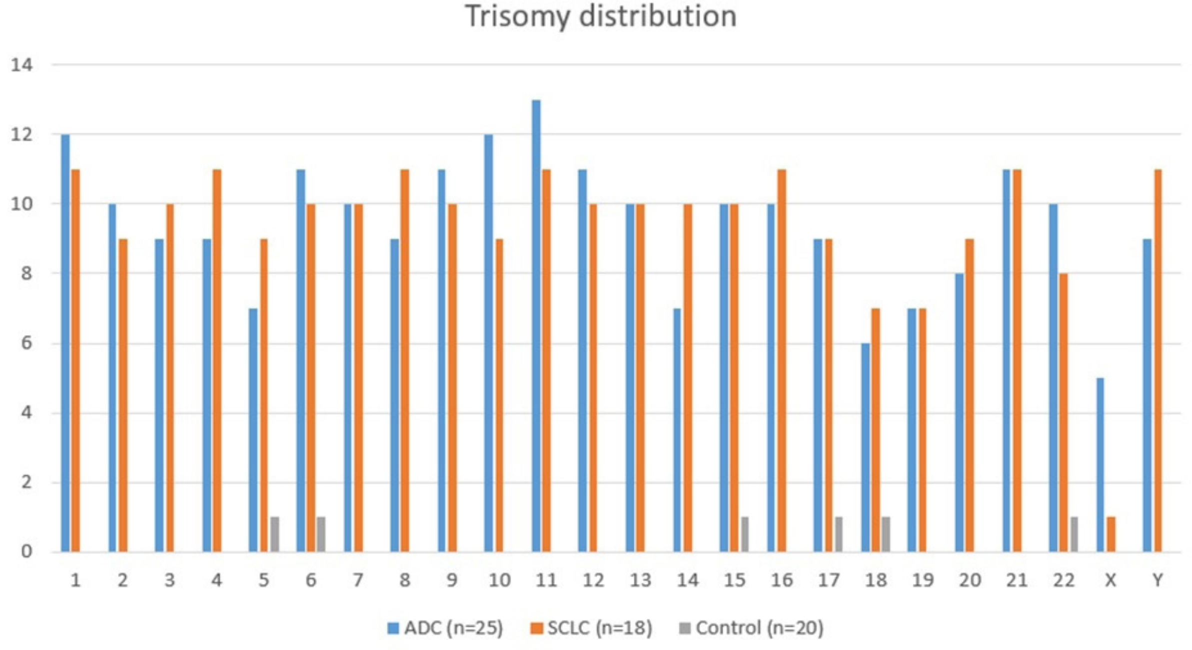

Figure 3. The distribution of trisomies over all the chromosomes between the SCLC and ADC cases.

Slides were prepared from cell suspensions and left to dry at room temperature. Following dehydration in subsequent alcohol series (70%, 85%, 100%) and air dried, the probes were applied on slides and denatured at 74°C for 2 min. Hybridization was carried out at 37°C for 18 h in a dark humidified cabin. After post-hybridization washes, the slides were counterstained with DAPI (4′-6′-diamidine-2-phenylindole) and examined under the fluorescence microscope (Olympus BX51, Tokyo, Japan). An average of 200 (range = 100-320) interphase nuclei were analyzed independently by two investigators. The cut-off value was calculated using the β inverse function in Microsoft Excel with a 95% confidence level on the control group's signals as 5%.

Statistical Analyses

The SPSS 20 package program was used for the statistical analysis, with $p<0.05$ being accepted as the statistical significance limit.

Mean, standard deviation, median, minimum, and maximum values are given in the descriptive statistics for continuous data, with percentage values presented in discrete data.

Chi-square is used to compare groups according to metastasis, stage, smoking status, gender, and FHIT deletion. The Mann-Whitney U test is used to compare the ages and FHIT deletions in the two patient groups.

RESULTS

Conventional Cytogenetics

The study examined 100 metaphases for the 18 SCLC, 25 ADC, and 20 control subjects and obtained good-quality metaphases. Karyotype formulas of the ADC and SCLC patients are provided in Supplementary Tables 1 and 2, respectively.

All the patients had clonal chromosomal abnormalities with composite karyotypes in both the SCLC and ADC groups. The clonal structural abnormalities observed in the series involved del(18)(p11) in six patients (2 SCLC, 4 ADC), del(6)(q15q21) in four patients (1 SCLC, 3 ADC), and del(22)(q12) in two patients (1 SCLC, 1 ADC). In addition to the recurrent abnormalities observed among patients, Table 2 also presents the clonal structural abnormalities that are unique to individual cases. No structural abnormalities were found in the control subjects.

A comparison of metastatic status and karyotype formulas from the ADC and SCLC cases are shown in Supplementary Tables 1 and 2, respectively.

Clonal numerical anomalies were detected in all patients from both patient groups. Monosomies of all chromosomes were observed in both patient groups and were the most prominent abnormalities in this study. Monosomy 22 (81% in 15 SCLC and 20 ADC) and monosomy 19 (77% in 15 SCLC and 18 ADC) were the most frequent abnormalities. Monosomy 3 was noted in 56% (8 SCLC and 16 ADC) of the cases. The control group had three cases of monosomy X, which was the only monosomy observed in more than one control group case. All monosomies except monosomy X were significantly higher in the study groups than in the control group ($p<0.05$), whereas no significant difference was found between the study groups ($p>0.05$). Clonal trisomies of all chromosomes were also observed in both patient groups. Trisomy 11 was the most recurring and was detected in 56% (11 SCLC and 13 ADC) of the cases. Trisomy of chromosome 3 was seen in 44% (10 SCLC and 9 ADC) of the cases. All trisomies occurred significantly more frequently than in the control group ($p<0.05$), but no significant difference was observed between the study groups ($p>0.05$). Figures 2 and 3 present the distributions of monosomies and trisomies for all chromosomes among the SCLC, ADC, and control cases. Figure 4 shows one example of a complex karyotype image of an ADC case.

FISH

Interphase FISH was applied to the peripheral lymphocyte preparations of 30 untreated ADC, 24 untreated SCLC, and 20 healthy control subjects using a custom-designed, quality-controlled FHIT (3p14.2) gene probe, with at least 200 cells being evaluated for each case. FHIT deletion was positive in 9 (30%) ADC and 7 (29%) SCLC patients. No significant difference was found between the ADC and SCLC groups ($p>0.05$). No significant difference was also found between the ADC and SCLC groups in terms of such anomalies as monoallelic and biallelic deletions, monosomy, or rearrangements ($p>0.05$). Monoallelic deletions were significantly higher in ADC and SCLC patients than in the control group ($p<0.001$ for both groups). Biallelic deletions were significantly higher in the ADC cases than in the control group ($p<0.05$). Monosomic cells were significantly higher in the ADC and SCLC patients than in the control group ($p<0.01$ and $p<0.001$, respectively). Rearrangements were significantly higher in the ADC and SCLC patients than in the control group ($p<0.05$ and $p<0.01$, respectively). No trisomic cells were observed in any group.

When comparing the patients according to their FHIT deletions and clinical variables such as stage, metastasis, smoking, gender, and age, no significant differences were found (Table 3). Figure 5 shows one example of a FHIT FISH image from an SCLC case.

DISCUSSION

In cases with no constitutional chromosomal abnormalities, many cancer-related chromosomal abnormalities are first seen in peripheral lymphocytes as constitutional abnormalities before being observed in tumor cells, such as in del(13q) in retinoblastoma, 3p abnormalities in renal cell carcinoma, del(5q) in colorectal carcinomas, or del(11p) in Wilms' tumor. In addition, some cancer cases have cancer-related chromosome anomalies in a small number of peripheral lymphocytes (11, 14, 23), with 3p deletions being prominent cytogenetic findings in SCLC and non-small cell lung (NSCLC) tumor tissues. These abnormalities have been reported rarely in the peripheral lymphocytes of SCLC patients (8, 11, 14, 15).

Because peripheral lymphocytes are easy to collect and process and are suitable for recurrent analyses, they would be valuable sources for predicting cancer development, prognosis, and future metastasis should cancer-related chromosomal abnormalities be scannable in this type of tissue. Efforts have also been made to evaluate this possibility in different cancers (23) apart from lung cancers (8, 11, 14, 15). However, more cases must be studied in order to be able to deduce the approach as being a feasible method.

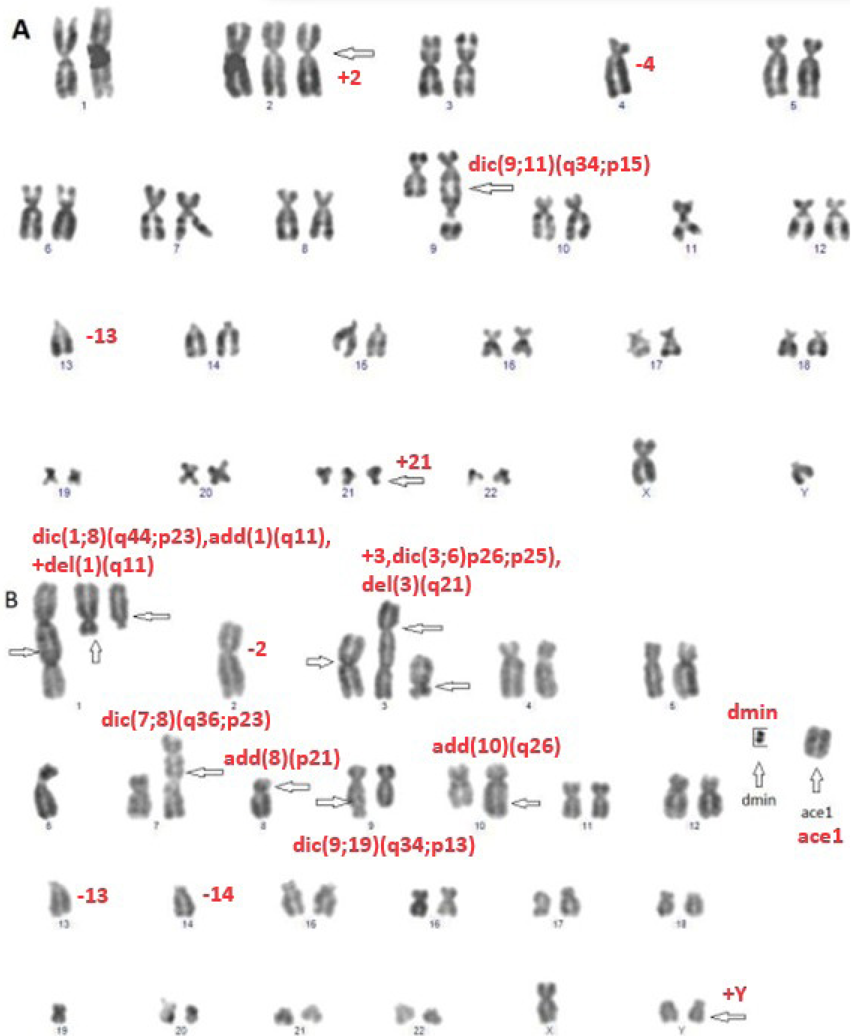

Figure 4. Two karyotype image examples of an ADC case (Case No. A2) containing numerical and structural abnormalities.
A. 46, XY, +2, -4, dic(9; 11) (q34; p15), -13, +21;
B. 43, XY, +Y, dic(1; 8) (q44; p23), add(1) (q11), +del(1) (q11), 2, +3, dic(3;6) (p26; p25), del(3) (q21), dic(7;8) (q36;p23), add(8)(p21), dic(9;19)(q34;p13), add(10)(q26), -13,-14, ace1, dmin

To enlarge the data on the subject, the current study has analyzed the peripheral lymphocytes of 24 untreated SCLC and 30 untreated lung ADC cases, alongside 20 healthy control subjects and observed complex karyotypes in both groups of cancer patients. Numerical abnormalities were observed to be more prominent than structural ones. Being present in 81% (15 SCLC and 20 ADC) of the cases, this study observed monosomy 22 as the most frequently occurring abnormality, followed by monosomy 19 at a rate of 77% (15 SCLC and 18 ADC). Monosomy 3 was noted in 56% (8 SCLC and 16 ADC) of the cases. All monosomies except for X, which was observed in three cases in the control group, were significantly higher than in the control group ($p<0.05$). However, no significant difference was observed between the two study groups ($p>0.05$).

This study found recurrent structural anomalies to occur in del(18)(p11) (14%; 4 ADC and 2 SCLC), del(6)(q15q21) (9%; 3 ADC and 1 SCLC), and del(22)(q12) (5%; 1 ADC and 1 SCLC). Structural chromosome 3 abnormalities were detected in only 2 (5%; 1 ADC and 1 SCLC) patients and occurred in del(3)(p22), t(3;5)(p25;q31) for the SCLC patient and add(3)(q29) for the ADC patient.

Abnormal karyotyped cells occurred at a rate of 1-10% in the control group, 13-91% in the SCLC group, and 18-75% in the ADC group ($p<0.05$). This discrepancy between the ratios of patient and control groups could indicate the genomic instability and heterogeneity of tumor cells. In fact, this abnormal-to-normal cell ratio in itself can be considered a better indicator of malignancy compared to looking for specific chromosomal abnormalities in peripheral blood. This index will

also cover the cells with nonclonal abnormalities that have previously been ignored as background noise and not included in the karyotype formulas but only recently were considered as significant indicators of genome instability (24).

A previous study reported cytogenetic results regarding peripheral lymphocytes in two SCLC cases (8). One of the cases involved a 57-year-old woman (Case 1) who had bone metastasis at the time of diagnosis and later developed a brain metastasis. The other patient was a 71-year-old man (Case 2), and the smoking history was positive for both patients. Blood samples were collected before the cytotoxic therapy. Case 1 had an extremely complex karyotype consisting of diploid and tetraploid clones heavy with structural and numerical chromosomal abnormalities. She had various chromosome 3 abnormalities, both clonal and nonclonal. This case was the main reason for planning to perform the current study. Case 2 had a less complicated karyotype without clonal chromosome 3 abnormalities, while nonclonal inv(3)(p14q29) was observed in two cells (one diploid, one tetraploid) in Case 1. Although the current study did not observe the same chromosome 3 abnormalities in its cases, a common breakpoint was observed: 3q29 was seen to be involved in two different inversions of 3 in the previous study and in one ADC case (patient A10) in the current study. The inversions with the 3q29 breakpoint that were observed in the previous study were inv(3)(p14q29) and inv(3)(q21q29), with inv(3)(p14q29) being observed in the one cell of Case 2, as well as the one diploid and one tetraploid cell of Case 1. Meanwhile, add(3)(q29) was the abnormality associated with the breakpoint 3q29 in the current study's ADC case for patient A10. No other common structural chromosomal abnormalities were observed between the two cases in the previous study or in the cases of the current study. As for numerical abnormalities, no trisomies had been found in the previous study, while some monosomies had been found in Case 1. Among these were monosomies 2, 8, 12, 16, and 17 in the diploid clone and monosomies X, 1, 7, 13, 17, 21, and 22 in the tetraploid clone. Monosomy 22 is one of the most frequent abnormalities of the current series, along with monosomy 19, with these being seen in 35 and 33 cases (81% and 77%), respectively. The previous study examined 63 cells in total for Case 1. Only six of these cells showed a normal karyotype, while 33 cells had at least one clonal abnormality and were included in the composite karyotype. Also, 24 cells carried nonclonal abnormalities. Overall, 90.4% of the cells were abnormal. However, the previous study investigated 100 cells for Case 2, with only 11 abnormal karyotyped cells (either clonal or nonclonal) being found. Therefore, this case would be indistinguishable from normal subjects when using peripheral blood cytogenetics if one of the nonclonal abnormalities is not inv(3)(p14q29), which is known as an abnormality associated with lung cancer.

Dave et al. reported cytogenetic results regarding the primary tumors and peripheral lymphocytes of 10 lung cancer cases (9 NSCLS and 1 SCLC) (14). They reported concordance between chromosomes altered in the lymphocytes and tumor cells of the same case, although sometimes the abnormality was able to differ. Moreover, they stated this to imply that "the susceptibility of particular chromosomes to break more frequently than others" is what is transmitted from the progenitor cells. However, they observed mainly numerical abnormalities in their work, with practically all chromosomes appearing altered. They concluded the genomic instability at the chromosomal level in the peripheral blood lymphocytes to correspond with tumors and "peripheral blood lymphocyte chromosomal

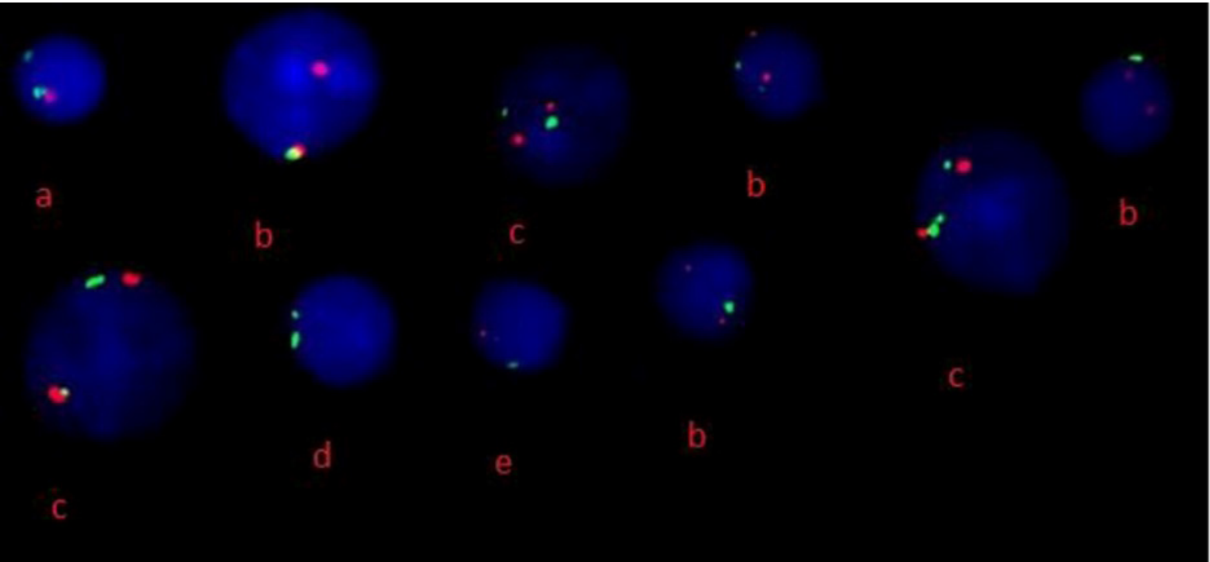

Figure 5. FISH image of an SCLC case (Case No. K9) containing (a): monoallelic (2G1R) deletion, (b) rearrangements (2R1G), (c) normal cell (2R2G), (d) biallelic (2G) deletion, and € monosomy (1R1Y). (Note: Green = centromere region of the 3rd chromosome, red = FHIT gene region of the 3rd chromosome)

analysis has a promising future in the genetic analysis of lung cancers" (14). Along with this comparative report between the tumor and peripheral blood of the same cases, Dave et al. also published the results of a broader cohort that examined only the peripheral lymphocytes of 96 untreated lung cancers and 74 normal control subjects in the same year. They declared in their report that more than 15% of their cases had structural or numerical chromosomal abnormalities for chromosomes 1, 3, 5, 7, 9, 12, 14, and 21 (11). These chromosomes had also been reported as being frequently involved in over 20% of the cases in their first-mentioned study (14). As for chromosome 3, their comparative study observed that, while chromosome 3 had been structurally rearranged in all 10 tumors, it had been rearranged in the peripheral lymphocytes in six cases (14).

De Fusco et al. reported chromosome aberrations of peripheral blood in seven SCLC cases. They found no chromosome 3 abnormalities but observed four aneuploid cells in one case: -Y in two cells, +5 and +12 in one, and +8 and +12 in another cell. Because +5, +8, and +12 were involved in the complex karyotype of the tumor tissue of the same patient and because the patient who had entered remission after treatment had relapsed and developed brain metastasis afterward, they commented that the aneuploid peripheral cells might have been circulating the tumor cells responsible for the metastasis (15).

When considering the cases in the literature alongside the current study, peripheral blood cytogenetics can be helpful as an indicator of chromosome instability with the observation of higher percentages of karyotypically abnormal -especially aneuploid- metaphases, as well as specific chromosomal abnormalities for the malignancy in question. However, quite a number of metaphases (100 if possible) need to be examined, and this is time- and effort-consuming in addition to also requiring expertise. Time and effort issues can be overcome to some extent through automatization when scanning the slides and capturing and analyzing the metaphases. However, finalizing the analyses will still require quite a lot of time and effort of an expert cytogeneticist. Therefore, conventional cytogenetics does not seem very practical for this purpose.

In addition to the cytogenetic analyses, this study performed FISH analyses to identify any possible submicroscopic deletions in the 3p14-21 critical region by targeting the FHIT gene in this region. FHIT is a tumor suppressor gene and a DNA caretaker. Loss of heterozygosity (LOH) in FHIT has been reported in previous studies and is considered an early alteration in lung cancer (5, 7, 25-27), with loss of FHIT expression causing genomic instability.

The current study detected FHIT deletion in about 30% of the patients (9 ADC and 7 SCLC), which was significantly higher than in the control group ($p<0.05$). Although the LOH of FHIT is reported in more than 50% of precancerous lesions and tumors, when considering that the material this study examined was peripheral blood, the rate in this study can be considered high. FHIT deficiency causes replication stress and DNA breaks. Replication stress can lead to micronucleus formation, aneuploidy, copy number alterations, and deletions at common fragile sites.

Owing to co-localization with FRA3B, the abnormal expression of FHIT boosts replication stress in this region, leading to FHIT deletions (28). FHIT loss causes genomic instability, leads to a mutator phenotype with a very high mutational rate, and accelerates tumorigenesis, progression, and metastasis. Because of these effects, FHIT mutations are considered as a driver mutation in cancer and could serve as a prognostic biomarker for many cancers (19, 26). Regarding the current study's rate using FISH, obtaining these deletions and mutations in circulating cells in the blood can possibly be considered significant. However, the FHIT status in the tumor tissue remained unclear in this study's patients due to being unable to access the tumor materials for a comparison. This can be considered as a limitation of the study. Further studies are needed for comparing FHIT status in blood and tumor samples and for assessing its potential as a biomarker.

In conclusion, the FISH technique could be useful for detecting FHIT loss in blood samples apart from tumor tissues. FISH is also capable of indicating aneuploidy as a marker of genomic instability. Present and future reports will enable the selection of recurring aneuploidies to scan with centromeric FISH probes along with relevant locus-specific probes to create a FISH panel for lung cancer.

Ethics Committee Approval: The study has been conducted in accordance with the Helsinki Declaration and was approved by the Cerrahpaşa Faculty of Medicine Medical Ethics Committee (Reference No. 83045809-604.01.02-A49).

Informed Consent: All patients and individuals in the control group having signed informed consent forms.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Y.T.A., A.C., N.B., H.G.O.; Data Acquisition- H.G.O., E.B.T., N.B.; Data Analysis/Interpretation- Y.T.A., A.C., N.B.; Drafting Manuscript- Y.T.A., N.B.; Critical Revision of Manuscript- Y.T.A., A.C., N.B.; Final Approval and Accountability- Y.T.A., A.C., N.B., H.G.O., E.B.

Conflict of Interest: The authors declare that they have no competing interests.

Financial Disclosure: This project was supported by the Istanbul University Scientific Research and Project Commission (Project No. 23831).

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Supplementary Table 1: Comparison of karyotype formulas with metastatic status of ADC cases		
ADC Case No	**Karyotype Formula**	**Metastasis Status**
A1	38~45,XY,-Y[6],-8[5],-9[3],-10[3],-11[5],- 12[5],-13[3],-14[3],-17[3],-18[4],-19[5], - 20[7],-21[6],-22[3][cp20]/18~28,X,+Y[2],+1[3],+9[2],+11[2],+13[2], -18[3][cp3]/46,XY[71]	Liver Met
A2	38~48,XY,+Y[2],-1[3],-2[4],+2[2],-3[3],-4[3],-5[3],del(6)(q15q21)[3],- 8[5],dic(9;11)(q34;p15)[2],+10[2],add(10)(q26)[2],+11[3],-13[4],+13[2],-15[3],- 17[6], -19[4],+19[2],-21[4],+21[3],- 22[4]+22[2],del(22)(q12)[2],ace1[6],dmin[5][cp27]/17~31,X,+Y[3],+2[2],+9[2],+13[2][cp3]/46, XY[55]	Bone Met
A3	38~45,XY,-8[8],-11[4],-14[4],-16[4],-17[4],-18[4],del(18)(p11)[2],-19[6],-20[8],-21[8], -22[6][cp30]/46,XY,del(6)(q15q21)[2]/46,XY[55]	-
A4	32~45,XX,-X[9],chtg(16)(q21)[4][cp12]/47,XX,+21[2]/46,XY[74]	-
A5	40~46,XX,-X[9],+Y[2],-2[3],-3[3],-6[3],-7[3],-10[3],- 15[3][cp15]/16~34,X,+1[3],+2[5],+3[4],+4[4],+5[6],+6[3],+7[4],+8[4],+9[4],+10[4],+1 1[6],+12[3],+13[3],-15[3],+15[3],+16[5],-17[3],+17[3],+19[2],+20[2],+21[4], -22[4],+22[2][cp10]/46,XX[68]	-
A6	35~45,XY,-Y[6],-4[4],-6[4],-7[5],-18[4],-21[3][cp14]/ 46,XY[78]	-
A7	36~45,XY,-5[3],-7[4],-10[6],-15[4],-16[5],-17[3],-22[7][cp17]/ 27~33,X,+1[3],+6[2],+7[2],+10[2],+15[2],+16[2],+17[2],+18[3] +19[2],+20[2],+21[2][cp3]/47,XY,+21[2]/4 6,XY[68]	-
A8	39~45,XY,-Y[4],-2[3],-6[3],-14[4],-16[4],-19[4],-20[5][cp15]/ 25~33,X,+2[3],+11[3],+12[2],+14[2],+15[3] [cp3]/46,XY[56]	Liver Met
A9	37~45,XX,-X[9],-2[3],-3[3],-5[3],-6[3],-7[3],-8[4],-14[3],-18[3],-19[5],-21[5], -22[6][cp25]/26~28,X,+6[2],+9[2],+10[2],+11[3],+12[4],+13[3],+15[3],+22[2][cp4]/46, XX[51]	Brain Met
A10	36~46,XX,-X[17],del(X)(p11)[2],-3[3],add(3)(q29)[4],-5[4],-6[4], -7[3],-8[5],- 10[6],-13[3],-14[3],15[3],-18[3],-19[5],- 20[3],-21[3],- 22[3][cp37]/47,XX,+X[3]/47~48,XX,+8[3],+ 10[2][cp3]/28~32,X,+1[2],+3[2],+7[2], +12[2],+17[2][cp2]/46,XX[52]	-
A11	38~47,XX,-X[21],-4[3],-5[4],- 6[6],del(6)(q15q21)[4],-7[3],-8[7],-9[6], -10[12],-11[6],-12[4],-13[6],-14[5],-15[5],- 16[7],-17[4],-19[4],-20[7],-21[7],-22[6][cp50]/18~33,X,+X[3],+1[3],+2[3],+3[2],+4[3],+5[2],+6[3],+7[3],+8[2],+9[4], +10[3],+11[3],+12[4],+13[3],+14[3],+16[5],+17[3],+18[4],+19[2],+20[5],+21[4], +22[4][cp 7]/46,XX[36]	-
A12	39~46,XY,inv(10)(q23q24)[5],del(14)(q24)[4],-18[4],-19[5],-20[4],-21[4],- 22[4][cp22]/27~32,X,+Y[2],+1[3],+4[2],+6[2],+7[2],+9[2],+12[2],+15[2],+16[2], +22[2][cp3]/46,XY[70]	Bone Met
A13	NMFE	-
A14	NMFE	Liver Met+ Bone Met

A15	35~45,XY,-X[6],-Y[6],-1[4],-2[13],-3[16],-4[9],-5[3],-6[16],-7[10],-8[15],-9[14], -10[9],-11[10],-12[13],-13[18],-14[14],-15[11],-16[11],-17[20],-18[4],-19[11], -20[9],-21[13],-21[4],- 22[9][cp56]/26~33,X,- X[5], +Y[6],+1[9],+2[5],+3[11],+4[8],+5[10],+6[3],+7[7],-8[4],+8[3],+9[5], -10[3],+10[6],-11[3],+11[9],+12[11],-13[5],+13[9],+14[7],-15[6],+15[4], -16[3],+16[8],-17[4],+17[5],+18[11],+19[5],+20[4],-21[6],+21[9],-22[3], +22[9][cp18]/46,XY[20]	-
A16	35~46,XX,-X[28],-2[9],-3[17],-4[6],-5[7],-6[8],-7[6],del(7)(p13)[3],-8[8],-9[4], -10[7],-11[4],-12[7],-13[4],-14[7],-15[11],-16[8],-17[9],-17[3],-18[9],del(18)(p11)[5], -19[20],-20[12],-21[9],-22[10][cp57]/21~33,X,-X[4],+X[2],+1[6],+2[4],- 3[3]+3[4], +4[3],+5[6],+6[6],+7[4],+8[3],+9[5],+10[5],+11[4],+12[4],+13[5], -14[4],+14[4],+15[7],+16[2],+17[3],-20[3],+20[4],+21[5],+22[6][cp12]/46,XX[26]	Esophagus Met
A17	35~50,XX,-X[6],+X[5],-2[6],-3[4],-4[8],-6[8],-7[5],-7[3],-8[8],-9[3],-10[4], +10[2]-11[4],-13[5],-14[3],-15[4],-20[12],-21[9],-21[4],-22[12][cp43]/46,XX[44]	-
A18	35~45,XY,-3[5],-4[3],-5[3],-7[3],-9[5],-10[10],-12[4],-14[6],-17[8],-18[4],-20[6], -21[9],-22[5][cp27]/46,XY[50]	Esophagus Met
A19	36~46,XY,-Y[4],-1[4],-3[4],-8[8],-11[4],-12[6],-16[6],-17[4], -18[4],del(18)(p11)[2],-19[6],-20[6],-21[6],-22[6][cp32]/46,XY[46]	-
A20	NMFE	-
A21	NMFE	Brain Met+Bone Met
A22	NMFE	Bone Met
A23	40~46,XY,-5[3],-8[13],-9[6],-13[7],-14[4],-15[4],-17[6],-18[4],-19[15],-20[6],-21[6], -22[8][cp43]/22~33,X,+Y[16],+1[9],-2[6],+2[5],+3[8],-4[3],+4[6],+5[6],+6[12], -7[4],+7[9],+8[2],+9[7],+10[6],-11[6],+11[5],-12[3],+12[6],-13[3],+13[8], -14[4],+14[3],-15[7],+15[6],+16[6],-17[5],+17[6],+18[5],-19[3],+19[3],+20[5], -21[3],+21[5],-22[6],+22[3][cp17]/46,XY[32]	Brain Met
A24	35~46,XY,-Y[3],-1[7],-3[7],-4[10],-5[11],-7[4],-9[8],-10[9],-11[4],-12[15],-13[8], -14[6],-16[6],-17[7],del(18)(p11)[5],-	-
	19[16],-20[8],-21[3],-22[11][cp50]/22~34,X,- X[7],+Y[11],+1[5],+2[6],+3[4],+5[4], - 6[4],+6[6],+7[5],+8[3],-9[6],+9[5],+10[6],+11[7],+12[6], 13[4],+13[6],+14[9],+15[10],+16[7],-17[3],+17[7],+18[6], del(18)(p11)[2],+20[3],-21[3],+21[4],+22[6][cp14]/46,XY[29]	
A25	40~46,XY,-X[3],-3[5],-6[4],-7[4],-10[4],-12[3],-13[6],-18[4],-19[5], -20[3][cp21]/46,XY[64]	-
A26	35~45,XY,-Y[3],-1[6],-3[6],-4[4],-5[7],-6[4],-7[4],-8[8],-9[7],-10[12],-11[5], -12[3],-13[7],-15[4],-16[11],-16[4],-17[10],-18[9],-19[4],-19[4],-20[12],- 21[12],-22[8] [cp42]/27~30,X,+Y[2],+1[2],+2[4],+4[2],+6[4],+7[2],+8[2],+9[2],+11[2],+12[2], +16[2],+17[2],+18[2],+19[2],+20[2][cp4]/46,XY[44]	-
A27	36~46,XY,-1[4],-3[4],-5[5],-6[4],-7[5],-8[7],-9[5],-10[7],-13[4],-14[5],-15[6], -17[4],-18[4],-19[5],+21[4][cp25]/46,XY[57]	-
A28	36~45,XY,-X[5],-Y[10],-2[5],-3[9],-4[9],-5[9],-6[6],-7[5],-8[15],-9[9],-10[11], -11[9],-12[7],-13[9],-14[9],-15[7],-16[9],-17[18],-18[10],-19[16],-20[19],-21[10], -22[18],-22[7][cp50]/46,XY[32]	-
A29	35~45,XY,-1[4],-3[4],-8[4],-10[4],-11[4],-12[8],-14[3],-15[3],-16[9],-17[8],-18[6], -19[4],-20[12],-21[13],-22[8][cp29]/28-34,X,+Y[3],+2[3],+3[4],+4[4],+8[4],+10[2], +11[3],+13[4],+15[2][cp4]/46,XY[31]	Bone Met

30	35~48,XY,+X[2],-Y[4],-1[6],-3[7],+3[6],-4[7],-5[5],-6[8],+6[2],der(7)t(7;7)(p10;q10) der(7)(q10;q10)[5],i(7)(q10)[7],-8[10],-10[4],-11[4],+11[2],-12[9],-13[10],-14[3], -15[7],-16[8],-17[3],-18[6],-19[6],-20[9],-21[13],-22[12],+22[3][cp34]/19~27, X,-X[5],+1[2],-2[4],+4[2],+5[2],-8[3],-13[3],+14[3],+16[3],-18[3],+21[3],+22[2] [cp6]/46,XY[23]	Brain Met

***ADC-adenocarcinoma; Met-Metastasis; NMFE- No Metaphase Found to Evaluate**

Supplementary Table 2: Comparison of karyotype formulas with metastatic status of SCLC cases

SCLC Case No	Karyotype Formula	Metastasis Status
K1	36~46,XY,-Y[7],-2[3],-4[3],-6[4],-8[3],-10[4],-11[4],-12[5],-15[6],-16[3],-17[3], -18[4],-20[6],-21[4],-22[3][cp23]/22~33,X,+Y[4],+1[3],+2[3], +3[3],+4[4],+5[3],+6[2],+6[2],+7[4],+8[2],+10[2],+11[2],+12[2],+13[2],+14[2], +16[3],+17[3],-18[3],+18[2],+19[2],+21[3],-22[5][cp6]/46,XY[61]	Liver Met+Muscle Met
K2	36~45,XY,-X[3],-1[3],-3[4],-5[3],-10[5],-11[3],-12[5],-13[4],-18[7],-20[3], -22[3][cp18]/28~34,X,+Y[2],+2[4],+3[2],+4[3],+5[2],+6[2],+7[2],+8[3], +10[4],+12[2],+15[2],+16[4],+17[4],+18[2],+19[4],+20[3],+21[2],+22[3][cp5]/46, XY[61]	-
K3	36~45,XY,-1[5],-2[3],-4[5],-5[7],-6[4],-7[4],-8[9],-9[3],-10[6],-11[3],-12[4], -13[4],-15[3],-17[7],-19[10],-20[3],-21[3],-22[7][cp27]/28~33,X,+Y[3],+1[3],+3[2], +5[3],+7[3],+9[2],+10[2],+11[2],+13[2],+14[3],+15[4],+16[2],+17[3],+19[4],+20[2], +21[2],+22[3][cp5]/46,XY[64]	-
K4	42~45,XY,-Y[4],-3[3],-10[5],-12[3],-20[3],-22[5][cp19]/46,XY[59]	Brain Met
K5	41~46,XX,-X[3],-8[3],der(18)del(18)(p11)del(18)(q12q22)[7][cp11]/40~45,XX, - 19[cp4]/46~47,XX,+8[cp2]/46,XX[69]	-
K6	NMFE	-
K7	NMFE	-
K8	NMFE	Brain Met
K9	35~52,XY,-1[4],-3[5],-6[4],-6[3],-7[4],-8[3],-9[4],-10[5],-11[4],-13[3], -14[4],del(18)(p11)[9],-19[3],-20[6],-21[10],-22[5],+22[2][cp32]/19~30,X,+Y[2],+4[2], +6[2],+8[2],+9[2],+11[3],+14[2],+15[2],+ 20[2],-22[4][cp4]/46,XY[51]	Brain Met
K10	NMFE	-
K11	NMFE	-
K12	35~46,XY,-X[5],-Y[7],-1[6],-2[7],-3[4],-4[6],-5[9],-6[6],-7[7],-8[16],-9[9], -10[11],-11[4],-12[18],-13[7],-14[7],-15[5],-16[11],-17[5],-18[8],-19[19], -19[4],-20[15],-20[3],-21[8],-22[9][cp47]/24~33,X, + Y[5],+1[4],+2[10],+3[5],+4[6],+5[6],+6[7],+7[4], -8[3],+8[2],+9[3],+10[5],+11[8],+12[7],+13[8],+14[4],+15[8],+16[5], -17[4],+17[4],+18[5],+19[4],-20[3],+20[6],+21[7],-22[5][cp14]/46,XY[21]	Head and Neck Met
K13	36~46,XY,-X[6],-1[4],-2[6],-7[4],-8[4],-9[6],-10[6],-11[7],-12[8],-13[4],-15[6], -16[5],-17[10],-18[13],-19[8],-20[6],-22[10][cp39]/33,X,+ Y[4],+1[4],+2[4],+3[3], +4[4],+5[2],+7[3],+9[2],+11[4],+12[3],+14[2],+15[3],+16[4],+17[3],+20[3],+21[2] [cp5]/46,XY[42]	Brain Met+Bone Met

K14	35~46,XY,-X[6],-Y[10],-1[14],-2[12],-3[10],-4[10],-5[14],-6[6],-7[6],-8[13],-9[6],-10[10],-11[4],-12[10],-13[12],-14[12],-15[12],-16[15],-17[10],-18[10],-19[20],-20[12],-20[6],-22[8][cp53]/20~23,X,+Y[4],+1[12],-2[4],+2[6],+3[13],+4[8],+5[5],-6[7],+6[4],-7[3],+7[7],+8[10],-9[7],+9[5],-10[4],+10[5],-11[3],+11[13],+12[9],-13[5],+13[6],-14[5],+14[7],-15[3],+15[4],-16[3],+16[10],-17[4],+17[8],+18[7],+19[7],-	-
	20[3],+20[5],-21[6],+21[3],-22[4],+22[5][cp18]/46,XY[28]	
K15	36~45,XY,-1[3],-3[7],-4[9],-5[7],-6[8],-7[5],-8[12],-9[7],-10[9],-12[5],-13[6],-14[14],-15[11],-16[14],-17[9],-18[9],-19[13],-19[4],-20[13],-21[14],-22[6][cp45]/28~32,X,+Y[11],+1[7],+2[5],+3[9],+4[6],+6[3],-7[3],+7[3],+8[8],+9[11],-10[4],+10[3],-11[3],+11[3],+13[8],+14[6],+15[6],+16[6],+17[5],+18[2],-19[5],-20[4],+21[5],+22[10][cp12]/46,XY[37]	-
K16	40~45,XY,-3[4],-5[6],-11[4],-14[4],-17[6],-19[7],-21[13],-22[6][cp36]/32~34,X,+Y[3],+1[2],+2[3],+8[2],+9[2],+11[3],+12[2],+13[3],+16[2],+20[3],+21[3][cp3]/46,XY[51]	-
K17	38~47,XY,-2[4],-7[7],-8[4],-9[3],-10[4],-11[4],-12[4],-14[6],-15[3],-19[4],-20[10],del(20)(q13)[4],-21[12],+21[4],-22[6][cp41]/30~34,X,+Y[6],-1[3],+1[3],+3[6],+4[3],+5[3],+6[2],+7[2],-8[3],+8[3],+9[3],+10[4],+11[2],+12[2],+13[3],+14[2],+15[2],+16[4],+18[3],+19[5],+21[3],+22[2][cp6]/46,XY[42]	Brain Met
K18	NMFE	-
K19	35~46,XY,-3[7],-5[8],-6[3],-8[8],-9[8],-10[8],-11[11],-11[4],-12[4],-13[3],-14[8],-15[6],-16[11],-17[8],-18[7],-19[8],-20[6],-21[4],-22[8][cp38]/21~34,X,- X[5],+Y[3],+1[3],+2[3],-3[3],+4[4],-6[3],+6[3],+7[5],+8[2],+9[2],+10[2],+12[3],+13[3],+14[3],+15[3],+17[6],+20[3],-22[3],+ 22[2][cp6]/46,XY[47]	-
K20	33~46,XX,-X[8],-2[3],-4[3],del(6)(q15q21)[5],-13[3],-13[3],-16[4],-17[5],-19[6],-20[3],-21[4],-22[5][cp32]/46,XX[51]	-
K21	37~47,XY,-Y[8],-6[6],-9[7],-10[3],-12[11],-13[8],-14[5],-16[3],-17[3],-18[6],-19[10],-20[6],-21[8],-22[5][cp36]/46,XY[55]	Bone Met
K22	38~50,XX,-X[5],+X[3],-6[4],-7[4],-12[6],-14[4],-17[8],-17[3],-18[6],-19[4],-22[4],del(22)(q12)[5],+mar2[3],+mar2x3 [2][cp29]/22~32,X,+ X[4],+1[2],+3[2]+4[2],+5[2],+6[3],+8[3],+9[2],+11[2],+12[3],+13[4],+15[3],+16[3],+19[2],+21[2][cp5]/46,XX[48]	-
K23	36~46,XY,-1[3],-2[4],t(3;5)(p25;q31)[2],-4[7],-5[4],-6[4],-7[7],-8[13],-9[8],inv(9)(p12q13)[71],-10[4],-11[6],-12[10],-14[4],-15[3],-16[8],-17[8],-18[7],-18[3],-19[9],-20[6],-21[8],-22[5][cp84]/30~33,X,+Y[4],+1[4],+2[4],+3[4],del(3)(p22)[2],+4[2],+5[6],+6[4],+7[4],inv(9)(p12q13)[4],+10[4],+11[6],+12[2],+13[2],+14[4],+16[2],+17[6],+18[2],+20[2],+21[4],-22[4],+22[2][cp6]/46,XY[9]	-
K24	38~45,XY,-Y[4],-5[3],-7[3],-12[3],-16[3],-17[4],-19[5],-21[5][cp10]/46,XY[70]	-

***SCLC-small-cell lung cancer; Met-Metastasis; NMFE- No Metaphase Found to Evaluate**