Differential Diagnosis of Multiple Primary and Intra-lung Metastasis of Lung Cancer by Multiple Gene Detection

Xu Liuyang
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Chen Jin
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Zeng Yupeng
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Ji Pan
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Zhuo Shuaishuai
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Li Xiao
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital
Zhihong Zhang (zhangzh@njmu.edu.cn)
Jiangsu Province Hospital and Nanjing Medical University First Affiliated Hospital

Research Article

Keywords: Multi-gene detection, Multi-focal lung cancer, Tumor heterogeneity, Targeted therapy

Posted Date: July 16th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-41708/v1

Abstract

Objective

To study the differential diagnosis of MPLC and IM by detecting the different lesions of the same patient. To explore the differences in prognosis between MPLC and IM, and to explore the factors affecting the prognosis of multi-focal lung cancer.

Methods

Fifty patients with multi-focal lung cancer were screened, and the relevant clinical information was noted; the patients were diagnosed by ACCP standard. Mutations of the lesions were detected by ARMS-PCR, and the detected genes included EGFR, ALK, ROS1, MET, KRAS, RET, HER-2, BRAF, NRAS and PIK3CA. The results of genetic testing were compared with those of ACCP standard diagnosis.

Results

We analyzed a total of 101 tumors from 50 patients. Classification based on gene testing contradicted the clinicopathologic diagnosis in 10 (20%) of the comparisons, identifying independent primaries in 6 cases diagnosed as metastasis and metastases in 4 cases diagnosed as independent primaries. Another 7(14%) tumor pairings were assigned an “equivocal” result based on gene testing. The results of gene testing of the remaining 33(66%) tumor pairings were consistent with the clinicopathologic diagnosis. The mutant heat map indicated that IM patients have a higher rate of mutation consistency than MPLC patients.

Conclusion

Multi-gene detection of multi-focal lung cancer has a certain auxiliary effect on the differential diagnosis of MPLC and IM, which can complement the clinical standards, but also has some limitations.

Background

The incidence and fatality rate of lung cancer are the highest among all cancers and are increasing each year[1]. Lung cancer is mainly divided into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for about 85% of all lung cancers. When the cancer occurs with more than one lesion, it is known as multi-focal lung cancer, and such cancers account for about 8% of all lung cancers. The cloning relationship between multi-focal lung cancer according to different lesion tumors can be divided into multiple primary lung cancer (MPLC) and lung cancer associated with pulmonary metastasis (intrapulmonary metastases, IM). MPLC can be again divided into simultaneous multiple primary lung

cancer (sMPLC) and heterogeneous multiple primary lung cancer, according to the time interval between the development of different lesions (metachronous multiple primary lung cancers, mMPLC)[2].

The concept of MPLC was first proposed by Beyreuther and others in 1924[3]. As lung cancer screening methods and diagnostic standards continue to improve, especially since low-dose spiral computed tomography (CT) has gradually become a mainstream screening method, it is reported increasingly frequently[4–5]. Differential diagnosis of MPLC and IM is particularly important for clinicians, because it has a direct impact on the TNM staging of lung cancer and plays a critical role in clinical management. For patients with MPLC, clinicians usually use local active treatment, while for IM patients, more systemic palliative care is used. Studies have shown that patients with MPLC have a better therapeutic response than those with IM[6].

Gene mutations occur before the proliferation of cancerous tissue cloning[7–8], that is, there is an essential difference between MPLC and IM in the origin of cloning; therefore, different mass in IM generally have the same type of gene mutation, and different mass in MPLC generally have different types of gene mutations[9–10]. This can be used as an important basis for multi-gene detection to assist in the diagnosis of multi-focal lung cancer.

In this study, the diagnosis and heterogeneity of multi-focal lung cancer were studied by multi-gene detection for clarifying the differential diagnosis of multiple primary lung cancer or lung cancer associated with pulmonary metastasis, and to understand the spatial heterogeneity of multi-focal lung cancer.

1. Materials And Methods

1.1 Clinical data

This study collected lung cancer specimens from patients admitted at the First Affiliated Hospital of Nanjing Medical University from 2014 to 2017, and selected 50 patients for this research. The conditions for inclusion were as follows: (1) Lung cancer was confirmed by pathological diagnosis; (2) The number of mass was greater than or equal to two, and sufficient specimens were available for study; (3) The patient did not receive any radiotherapy, chemotherapy or targeted treatment before surgery.

Exclusion criteria was based on lack of clinical data. Clinical data included the following: patient's name, gender, age, smoking history, cancer occurrence time, location, size, histological diagnosis, lymphatic metastasis, infiltration, genetic examination/no genetic examination, genetic status, and the result of follow-up.

We diagnosed all these cases based on ACCP criteria to distinguish between MPLC and IM.

1.2 Patient follow-up

The 50 patients were contacted to follow up their current physical condition by telephone, whether they had received targeted treatment and its efficacy, and whether they had recurrence.

1.3 Multi-gene detection

The main experimental method used in this study is ARMS-PCR.

1.4 Detection methods

1.4.1 Paraffin slides

One HE slide and eight regular slides for each paraffin block were used.

1.4.2 Pathological Assessment

The content of tumor cells was assessed by two experienced pathologists. Assess the distribution of whole tumor tissue under a low power lens. If there is an obvious tumor-free area, mark it out with a marker pen and remove the part from all paraffin slices to be tested. Select 5 fields of view (including top, bottom, left, right, center field of view) for slices at high magnification, count cells continuously for 30 cells at each field of view, combine the count results of 5 fields of view, and calculate the average tumor cell content as the tumor cell content of the sample sent for testing. The slice meets the test criteria when the tumor cell content in each slice is greater than 1%. If the views of the two pathologists are not in agreement, the results will be evaluated by the third pathologist.

1.4.3 Extraction and quality control of nucleic acids

DNA and RNA extraction was carried out using the ADx-FFPE DNA extraction kit and the ADx-FFPE RNA extraction kit (AD Biology, cargo number 8.02.24101X036G and 8.02.23501X036G), respectively. We used NanoDrop to quality control the extracted DNA and RNA. when ratio of OD260/280 was more than 1.7 and less than 2.0, the sample was qulified and required until the next genetic test can be carried out.

1.4.4 Gene mutation detection

Ten genes were detected using the ADx-5 gene mutation detection kit and the ADx-MET gene exon 14 missing mutation detection kit(AD Biology, cargo number 8.0126301w006A).

1.5 Statistical methods

The data were statistically analyzed using SSPS18.0 software. The survival curve (Kaplan-Meier) was used for correlation survival analysis.

2. Results

2.1 Patient data

Of the 50 patients enrolled in the group, 31 (62%) were women and 19 (38%) were men. The age range was 33–87 years. Forty-nine cases (98%) had two lesions, and one case (2%) had three lesions. A total of 101 tumors were detected, including 17 in situ cancer (16.83%), 68 in the T1 stage (67.33%), 8 in the T2 stage (7.92%), and 2 in the T3 stage (1.98%). There were six tumors in the T4 stage (5.94%). All 50 patients had simultaneous tumors, of which 48 were lung adenocarcinoma (96%), 2 were lung squamous cell carcinoma (4%), and there were no cases of adenocarcinoma lesions and squamous cell carcinoma lesions at the same time. In 14 cases, tumor occurred on the bilateral side, while in 36 cases, tumor occurred on the same side. Tumors were of the same histological types in 20 cases and of different histological types in 30 cases. The details are provided in Table 1.

Table 1
Clinical data summary table of enrolled patients

Factors		**Number(%)**
Gender	Male	19(38%)
	Female	31(62%)
History of smoking	Yes	11(22%)
	Never	39(78%)
Histologic type	ADC	48(96%)
	SCC	2(4%)
Number of lesion	Two	49(98%)
	Three	1(2%)
Location	Similar lobe	13(26%)
	Different lobe in similar side	23(46%)
	Different side	14(28%)
Metastasis	No	40(80%)
	Lymph node metastasis	10(20%)
PS: One patient had three lesions, two of which were on the same side of the same lobe, and the other was on the same side of the different lobe.		

2.2 Diagnostic results based on the ACCP standard

We diagnosed these 50 cases according to the diagnostic criteria for multiple primary lung cancer and lung cancer associated with pulmonary metastasis proposed by ACCP in 2007. Of the 49 cases with double lesions, 34 cases were diagnosed as IM and 15 were diagnosed as MPLC. The same patient had 3 lesions on the lung, all histological morphology of which were consistent. Two of the lesions on the same lobe were IM, and the third lesion in the other lobe was an independent primary lesion.

2.3 Gene testing results

Of the 101 tumors detected, 25 showed EGFR L858R mutations, 26 had EGFR 19th exon missing, 6 had EGFR 20th exon insertion, 7 had HER-2 mutations, and 4 had ALK mutations. There was a single tumor with NRAS mutations, 3 with KRAS mutations, 1 with RET mutations, one with BRAF mutations, one with EGFR 19th exon missing and KRAS mutations, and 26 tumors showed wild-type gene (as shown in Fig. 1). Among the cases with two lesions, 12 cases showed gene mutations of the same type;19 cases showed gene mutations of different types; 10 cases showed a mutation in one lesion but no mutation in the other lesion; and no mutation was detected in lesions of the remaining 8 cases. In the one case with three lesions, two tumors in the same lobe had the same gene mutation, i.e., missing EGFR 19th exon, and the tumor in the other lobe had a wild-type gene. In cases with two lesions with missing EGFR 19th exon, we conducted a sequencing analysis to determine the specific sites of their absence, and the results showed that one patient had two lesions of p. Del E746_s752InsV (c.Del2237_2255InsT), while in the remaining patients, the missing sites were E746_A750.

2.4 Comparison of the diagnostic results based on ACCP standard with the results of genetic testing

Comparison of the results of clinical diagnosis with the results of gene detection showed that 13 cases had the same types of gene mutations, of which 6 cases were diagnosed as MPLC and 7 as IM by ACCP standard; 18 cases had different types of gene mutations, of which 16 cases were diagnosed as MPLC and 2 cases were diagnosed as IM by ACCP standard. Gene mutation was detected in one lesion, while in another lesion, wild-type gene was detected in 11 cases, of which 9 cases were diagnosed as MPLC and 2 cases as IM by ACCP standard diagnosis. In two lesions, wild-type gene was detected in 7 cases, of which 3 cases were diagnosed as MPLC and 4 cases as IM with ACCP standard diagnosis, thus leaving 1 case of three lesions, in which two lesions were diagnosed as intra-lung metastatic carcinoma with the same type of gene mutation and the third lesion was the primary focal lesion with wild-type gene as detected by ACCP. In 33 cases, the results of gene testing were consistent with the differential diagnosis of ACCP standard; in 10 cases, the results were inconsistent, while the results could not be verified in 7 cases.

2.5 Relationship between clinical characteristics and gene mutations in patients with multi-focal lung cancer

In this study, the incidence of gene mutations in men and women with multi-focal lung cancer was 79.8% (15/19) and 90.3% (28/31), respectively, and there was no statistically significant difference between the two groups ($P > 0.05$). The incidence of gene mutations in those with a non-smoking history was 92.5% (37/40), and the incidence of gene mutations was 60% (6/10) in those with a history of smoking, and the difference between the two groups was statistically significant ($P < 0.05$). The incidence of gene mutations in patients with squamous cell carcinoma was 0% (0/2), and the incidence of gene mutations was 89.6% (43/48) in patients with adenocarcinoma, and the difference between the two groups was statistically significant ($P < 0.05$). The details are provided in Table 2.

Table 2
Relationship between gene mutation and clinical features in patients with multi-focal lung cancer

Factors	Number(n)	Number of patients with mutations (n)	Positive rate	χ^2	P
Gender				0.497	0.404
Male	19	15	78.9%		
Female	31	28	90.3%		
History of smoking				4.578	0.023
Never	40	37	92.5%		
Yes	10	6	60%		
Histologic type				6.439	0.017
ADC	2	0	0%		
SCC	48	43	89.6%		

2.6 Patient follow-up results

After telephonic follow-up, the prognostic information of 39 patients was obtained, and 11 patients missed their interview. Of the 39 patients, 3 died, 5 were targeted and their condition was stable, 5 had a relapse, and 28 did not use targeted therapy and their condition was stable. Patients with EGFR 19th exon deleting were treated with gefitinib. Those with the absence of EGFR 19th exon deleting in one lesion and with the mutation of EGFR 21st exon in another lesion were treated with Icotinib and Gefitinib. Those detected to have the mutation of ALK in each lesion were treated with Crizotinib. Those with the insertion of EGFR 20th exon in one lesion and the mutation of EGFR 21st exon in another lesion were treated with Gefitinib.

2.7 Realtionship of concordance rate of mutations of different lesions between MPLC and IM

We drew a mutant heat map based on clinical data and test results, as detailed in Fig. 2. From this diagram we established that compared with MPCL patients, IM patients have a higher rate of mutation consistency.

2.8 Relationship between gene mutations and lymph node metastasis and prognosis in patients with multi-focal lung cancer

The relationship between gene mutation and prognosis was analyzed using a survival curve: the difference of prognosis between patients with 10 gene mutations and those with wild-type genes patients

was statistically significant (P = 0.002), and the DPS of patients with 10 gene mutations was longer than that of patients with wild-type gene. The details are provided in Fig. 3A.

The relationship between lymph node metastasis and prognosis was analyzed by survival curve: patients with lymph node metastasis had a statistically significant difference in prognosis from those with no metastasis of lymph nodes (P = 0.006), and the DPS of patients with no metastasis of lymph nodes was longer than that of patients with lymph node metastasis. The details are provided in Fig. 3B.

2.9 Survival analysis of MPLC and IM patients according to ACCP diagnostic standard

The survival curve indicated a statistically difference (P = 0.038), implying that DFS in patients with IM was worse than in patients with sMPLC. The details are provided in Fig. 3C.

2.10 Relationship between mutations and prognosis of different lesions in patients with multi-focal lung cancer

According to the mutations of different lesions, the patients with multi-focus lung cancer were divided into three categories: no mutations were detected in each lesion; mutations were detected in each lesion; and mutations detected in each lesion were the same or different. The survival curve showed a statistically significant difference (P = 0.002). This implies that DFS in patients with different mutations was better than that in other patients. The details are provided in Fig. 3D.

3. Discussion

The purpose of this study was to investigate the feasibility of multi-gene detection in the differential diagnosis of multi-focal lung cancer. In the 50 cases we studied, 33 cases (66%) had consistent genetic test results with ACCP standard diagnosis, while 10 cases (20%) were inconsistent, and 7 cases (14%) results could not be validated. Within those 10 cases, 6 were diagnosed with MPLC by the ACCP standard but the same type of mutation was found in different lesions, 2 were diagnosed with IM by the ACCP standard but the mutation types were different, and 2 were diagnosed as IM by ACCP standard but mutation was detected in one lesion while no mutation was detected in another lesion.The same result was observed by Nicolas Girard ET, where inconsistent results were observed for 7 (32%) of the 22 cases [11]. Multi-gene detection of multi-focal lung cancer is more scientific. The heat map indicates that the same clone source mutation is related to IM, and different clone source mutations are related to MPLC.

In general, if the results of gene detection of two lesions in the lungs are the same, we consider that one of the lesions was metastatic. However, if the results of gene detection of two lesions in the lungs are different, we consider that both lesions are primary. In special circumstances, if the results of gene detection of two lesions in the lungs are the same, it is also possible that both lesions are primary. This is related to the type of mutation detected. If the mutant type of mutation in the population is very low, both lesions have a very low probability of mutation, and the two lesions are considered to be intra-lung metastasis. In this study, one patient had two lesion mutation types of HER-2, while HER-2 had a 1–2%

mutation rate in Asian lung cancer patients; therefore, the patient was considered IM. If a certain mutation type has a higher proportion in the population, it is also possible to have multiple primary lesions. In this study, two patients had two lesions with EGFR L858R mutations at the same time; three patients had EGFR 19th exon missing at the same time; the EGFR mutation rate in Asian lung cancer patients is 50%, of which 85%-90% EGFR was 19th exon missing and L858R mutation. Therefore, even if the two lesion mutations are consistent, it is possible to be independent of each other; at this time, it is necessary to combine clinical information to make a comprehensive judgment, such as the degree of similarity of histological type, the lobe in which the lesion is located, whether there is infiltration or lymph node metastasis. In addition, studies have shown that there is a certain inconsistency between the primary and metastatic lesions of lung cancer; hence, the genetic detection results of two lesions are inconsistent and may be IM, which is related to the type of gene mutation detected: if there are two different types of mutations and the transfer probability is very small, then the two lesions are considered to be independent of each other. If one of the lesions is detected as wild type and another is detected to be a mutant, it is also possible that these two lesions are metastatic [(12)]. The researchers believe that this is because the primary focus has a small number of mutant cells with strong metastasis ability, and because of this small amount, we cannot detect mutations in the primary focus; however, in the metastases region, the cell proportion is higher so that mutations are detected[13]. In this case, we need to combine clinical information to make a judgment such as the lung lobe of the lesions, the condition of lymph node metastasis, and the condition of tissue infiltration. In this study, two lesions in one patient were in the lower right lung, and the tissue morphology was diagnosed as intra-lung metastatic carcinoma according to the ACCP Standard, but according to the results of genetic tests, one of the two lesions had NRAS mutation and the other lesion had wild-type gene, which was in line with the above findings.

There were seven cases with different histological types of cancer stove but the same types of gene mutations. This fining indicates that the two lesions of tumor originated from the same tumor stem cell. The difference in histological morphology of the lesions may be caused by the differentiation during the development of the tumor stem cell. It also reflects the heterogeneity of the primary lesion and the metastatic lesion. According to ACCP standard, most of the multi-focus lung cancers with different histological morphology are diagnosed as MPLC. The reason is that the clinicians may ignore the existence of tumor heterogeneity between primary and metastatic tumors. This may also be one of the reasons why the clinical diagnosis results that rely on ACCP standards in this study are inconsistent with the results of genetic testing.

The identification of MPLC and IM has important guiding significance for clinical decision-making. According to the eighth edition of the international staging of lung cancer, if a patient has two or more nodules in the same lung lobe, his lung cancer stage is T3, if a patient has two or more nodules in different lung lobes on the same side of the lung, then his lung cancer stage is T4, and if a patient has two or more nodules in different side lungs, then his lung cancer stage is M1a. Therefore, MPLC is often ⅡB to IV stage in clinical staging, while the treatment of lung cancer in ⅡB to Ⅲ stage is generally

dominated by conservative radiotherapy and chemotherapy. Studies have shown that lymph node metastasis is a significant independent prognostic factor for MPLC patients, with five-year survival rates of 15.6% and 52.5%[14] in lymph node metastatic and non-metastatic patients, respectively. In this study, DFS of patients with MPLC was better than those with IM, DFS of genetically mutated patients was better than that of patients with wild-type gene, and DFS of patients without lymph node metastasis was better than that of patients with lymph node metastasis. Therefore, clinicians need to refer to the type of genetic mutations of each lesion when their patients have multi-focus lung cancer), rather than simply staging through nodule position, in order to provide better and more accurate treatment. Based on this study, we summarized the process of identifying MPLC and IM. The details are provided in Fig. 4.

The heterogeneity of tumor includes the heterogeneity of primary and metastatic lesion in the same patient, the heterogeneity within the same tumor, the heterogeneity of different primary lesions in the same patient, and the heterogeneity between different lesions in different patients. The heterogeneity of primary and metastatic lesion is mainly reflected in the difference of tissue morphology and the difference of gene mutation types or loci. Both the lesions in one patient were in the upper left lung, of which one was adenocarcinoma dominated by the glandular bubble type, and the other was adenocarcinoma dominated by the wall type. Thus, although the ACCP standard diagnosed MPLC, two lesions were have L858R mutations through genetic testing; hence, they were likely to have the same cloning origin. This reflects the heterogeneity of the histological of the primary lesion and the metastatic lesion. Both of the lesions in one patient were in the upper right lung, and the histological type was micro-invasive adenocarcinoma; thus, it was diagnosed as IM by the ACCP Standard, but the gene test revealed that one lesion was HER-2 positive and the other lesion was had missing EGFR 19th exon. This shows that there is an essential difference between the two in the origin of cloning, and this patient should be diagnosed to have MPLC. This reflects the heterogeneity between the primary lesion and the metastatic lesion. After discussion and analysis, we believe that the following reasons explain the heterogeneity between primary and metastatic lesions of lung cancer: sensitivity difference of the detection method[15]; heterogeneity of the internal variation of the sample itself[16]; evolution and development of the mutation state of the primary and metastatic lesion[17]; number of tumor cells and non-tumor cells in tumor samples[18].

In this study, one patient had a lesion that exhibited both the absence of EGFR 19th exon and the presence of KRAS mutations. Studies have shown that mutations in the KRAS gene and EGFR gene in NSCLC are often mutually exclusive[19], but other studies have also found cases where the two coexist (2/85), with limited benefits from TKI and poor prognosis[20]. We believe there is some rejection of the KRAS gene mutation and the EGFR gene mutation, but this exclusion is not absolute and may be related to genetic differences between races.

Because testing can guide the diagnosis of multi-focal lung cancer, it can guide clinicians in the targeted treatment of patients. The ten genes in this study had corresponding targeted drugs, of which the most studied and widely used were EGFR-TKIs for EGFR mutations. EGFR mutations (57.4%) were found in 58

of the 101 tumors in this study, which is slightly higher than the EGFR mutation rate (40–50%) in NSCLC in East Asian people, and the possibility of EGFR mutations was not ruled out in patients with multi-focal lung cancer. In addition, the data showed that the proportion of other mutations were as follows: HER-2 mutations in seven cases (6.9%); ALK mutations in four cases (4%); four cases of KNRAS mutations (4%); one case of NRAS mutations (1%); one case of RET mutations (1%); one case of BRAF mutations (1%); one case (1%) of ROS1 mutations. For patients with positive mutations, clinicians can recommend appropriate targeted drugs, which is significant for patients. Follow-up data showed that a total of five patients had targeted therapy and their condition was effectively controlled. We also found that in patients with two lesions with different EGFR mutations, the use of Gefitinib and Icotinib treatment was significant, and studies have shown that the first generation of EGFR targeted drugs, such as Gefitinib and Icotinib, are the best choice for patients with more than two mutations.

According to statistical analysis, 10 patients with gene mutations had longer DPS than wild-type patients, and DPS in patients with no metastasis of lymph nodes was longer than those with lymph node metastasis, suggesting that gene mutations and lymph nodes were important factors for good prognosis of multi-focal lung cancer. This helps to guide clinicians to adopt more appropriate treatment options for patients, thus maximizing their benefits.

To sum up, the differential diagnosis of MPLC and IM should not be confined to the analysis of clinical data, and the results of genetic testing should also be taken into account. The differential diagnosis of multi-focus lung cancer by traditional multi-gene detection methods such as ARMS-PCR has advantages of cheaper price, simpler process, easier interpretation of results, and higher sensitivity. More importantly, if a sensitive mutation is detected, targeted therapy can be carried out if necessary, which is of great help to the patient's prognosis.

Declarations

Ethics approval and consent to participate

Animal and human experiments are not involved in this article.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and material

The datasets used or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declared that they have no conflicts of interest to this work.We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

Funding

The authors were supported financially by provincial key projects201810312017Z.

Authors' contributions

Xu Liuyang and Chen Jin contributed equally to this work.

Acknowledgements

Xu Liuyang, Chen Jin: Conception and design of the study,acquisition and interpretation of data, drafting the article, final approval ofthe version to be published. Li Xiao, Zhang Zhihong: Acquisition of data, analysis and interpretation of data, revising the article, final approval of the version to be published. Zeng Yupeng, Zhuo Shuaishuai, Ji Pan: Conception and design of the study, analysis and interpretation of data, drafting and revising the article, final approval of the version to be published.

References

1. Alzahouri K,Martinet Y,Briancon S,et a1.Staging practices of primary non—small—cell lung cancer: a literature review [J].Eur J Cancer Care(Eng1),2006,15(4):348–354.
2. Celine Mascaux MW, Wynes. Yasufumi Kato,et al.EGFR Protein Expression in Non-Small Cell Lung Cancer Predicts Response to an EGFR Tyrosine Kinase Inhibitor – A Novel Antibody for Immunohistochemistry or AQUA Technology.Clin Cancer Res. 2011 December 15; 17(24): 7796–7807.
3. Beyreuther H.Multiplicitat von Carcinomen bei einem Fall von sog.“Schneeberger” Lungenkrebs mit Tuberkulose.Virchows Arc,1924,250(1/2):230–243.
4. Xue X, Liu Y, Pan L, et al. Diagnosis of multiple primary lung cancer: A systematic review[J]. J Int Med Res. 2013;41(6):1779–87.
5. Eberth JM, Qiu R, Adams SA, et al. Lung cancer screening using low-dose CT: The current national landscape[J]. Lung Cancer. 2014;85(3):379–84.
6. Warth A, Macher-Goeppinger S, Muley T, et al. Clonality of multifocal nonsmall cell lung cancer: Implications for staging and therapy[J]. Eur Respir J. 2011;39(6):1437–42.
7. Sherwood J, Dearden S, Ratcliffe M, et al. Mutation status concordance between primary lesions and metastatic sites of advanced non-small-cell lung cancer and the impact of mutation testing methodologies: a literature review.[J]. Journal of Experimental Clinical Cancer Research. 2015;34(1):92.

8. Vignot S, Frampton GM, Soria JC, et al. Next-generation sequencing reveals high concordance of recurrent somatic alterations between primary tumor and metastases from patients with non-small-cell lung cancer.[J]. J Clin Oncol. 2013;31(17):2167-U158.
9. Girard N, Deshpande C, Azzoli CG,et al.Use of epidermal growth factor receptor/kirsten rat sarcoma 2 viral oncogene homolog mutation testing to define clonal relationships among multiple lung adenocarcinomas:Comparison with clinical guidelines.Chest,2010137(1): 46–52.
10. Van Rens MT, Eijken EJ, Elbers JR,et al.p53 mutation analysis for definite diagnosis of multiple primary lung carcinoma.Cancer,200294(1): 188–196.
11. Girard N, Ostrovnaya I, Lau C, et al. Genomic and Mutational Profiling to Assess Clonal Relationships Between Multiple Non-Small Cell Lung Cancers[J]. Clinical Cancer Research An Official Journal of the American Association for Cancer Research. 2009;15(16):5184.
12. Sun L, Zhang Q, Luan H, et al. Comparison of KRAS, and EGFR, gene status between primary non-small cell lung cancer and local lymph node metastases: implications for clinical practice[J]. Journal of Experimental Clinical Cancer Research. 2011;30(1):30.
13. Sun L, Zhang Q, Luan H, et al. Comparison of KRAS and EGFR gene statuses between primary non-small cell lung cancer and local lymph node metastases and their clinical significance[J]. Chinese Journal of Clinical Oncology. 2012;102(36):971–84.
14. Chang YL, Wu CT, Lee YC. Surgical treatment of synchronous multiple primary lung cancers: Experience of 92 patients[J]. Journal of Thoracic Cardiovascular Surgery. 2007;134(3):630–7.
15. Ellison G, Donald E, McWalter G, Knight L, Fletcher L, Sherwood J, et al. Acomparison of ARMS and DNA sequencing for mutation analysis in clinical biopsy samples. J Exp Clin Cancer Res. 2010;29:132.
16. Tang X, Varella-Garcia M, Xavier AC, Massarelli E, Ozburn N, Moran C, et al. Epidermal growth factor receptor abnormalities in the pathogenesis and progression of lung adenocarcinomas. Cancer Prev Res(Phila). 2008;1:192–200.
17. Takahashi K, Kohno T, Matsumoto S, Nakanishi Y, Arai Y, Yamamoto S, et al. Clonal and parallel evolution of primary lung cancers and their metastases revealed by molecular dissection of cancer cells. Clin Cancer Res. 2007;13:111–20.
18. Dufort S, Richard MJ, Lantuejoul S de FF. Pyrosequencing, a method approved to detect the two major EGFR mutations for anti EGFR therapy in NSCLC. J Exp Clin Cancer Res. 2011;30:57.
19. Pao W, Wang TY, Riely GJ,et al. KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib□J□. PLoS Med. 2005;2(1):e17.
20. Dandan DONG, Gang XU, Fanghua LI,et al. Investigation of EGFRã□□KRAS Mutations and ALK Protein Fusion Status in Lung Adenocarcinoma Patients.The Practical Journal of Cancer, September 2017,Vol32,No.9.

Figures

Fig 1. Distribution of different gene mutations in lung cancer

Figure 1 shows the distribution of different gene mutations in lung cancer. In this study, 50 patients with a total of 101 tumors were examined, one of which had both EGFR 19 exon deletion and KRAS mutation.

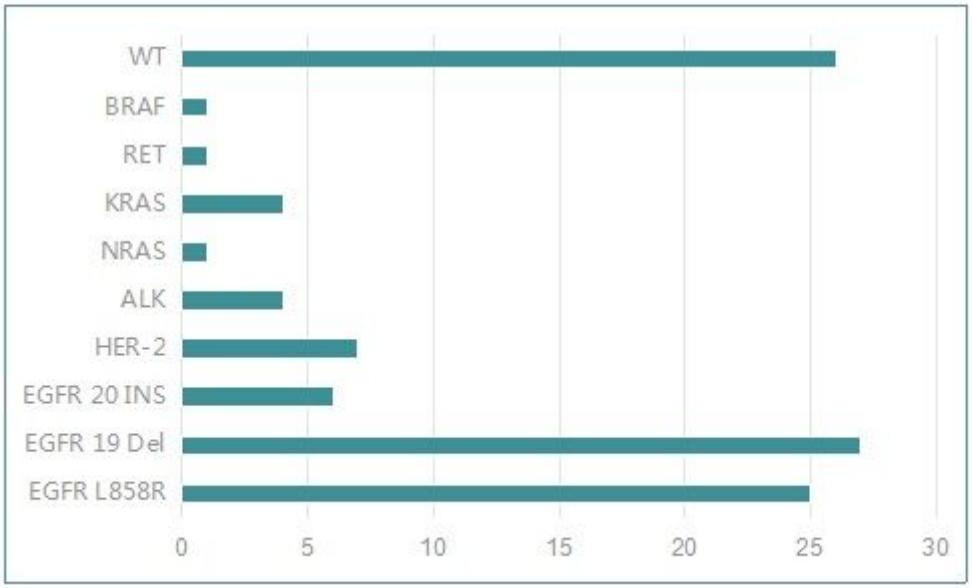

Figure 1

Figure 1

Fig 2. Mutant heat map based on clinical data and test results

Figure 2 contains the histological classification, stage, smoking, existence, mutation type and SNV concordance of the 50 patients in our study.

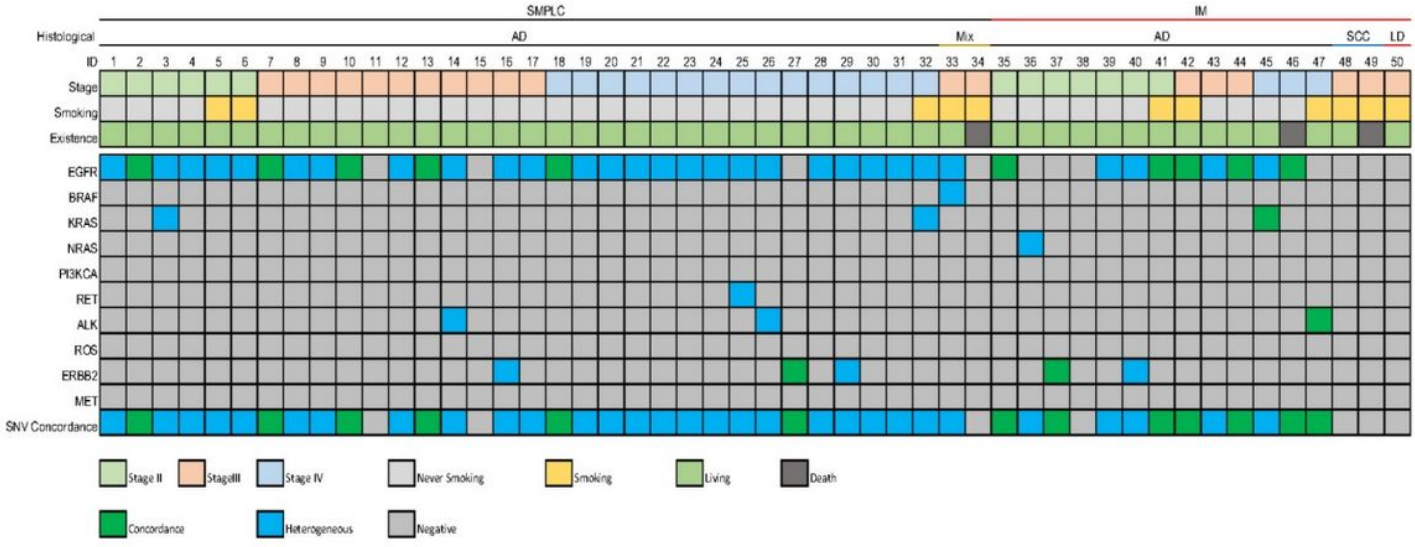

Figure 2

Figure 2

Fig 3A. KM Plot of MPLC by Mutation Status

Figure 3A shows the relationship between gene mutation and prognosis was analyzed using a survival curve.

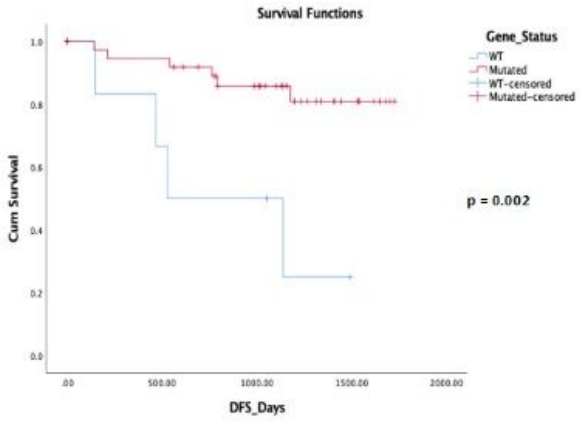

Fig 3B. KM Plot of MPLC by lymph node metastatic status

Figure 3B shows the relationship between lymph node metastasis and prognosis was analyzed by survival curve.

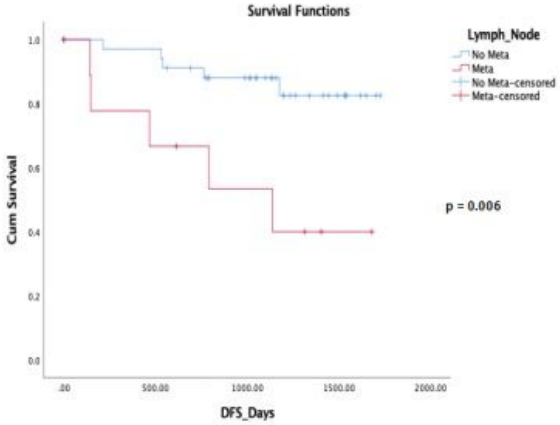

Fig 3C. KM Plot of MPLC

Figure 3C shows the survival analysis of MPLC and IM patients according to ACCP diagnostic standard.

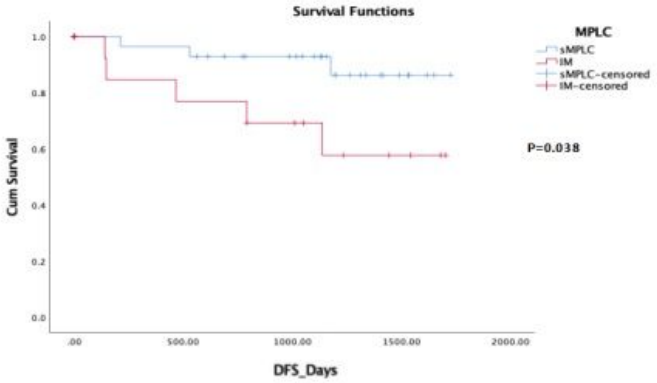

Fig 3D. KM Plot of MPLC by SNV Concordance

Figure 3D shows the survival analysis of patients with different states of mutations of their different lesions.

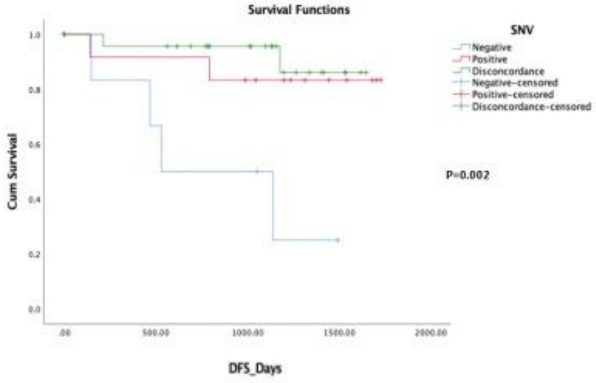

Figure 3

Figure 3

Fig 4. Process of identifying MPLC and IM

Figure 4 shows the process of identifying MPLC and IM based on this study.

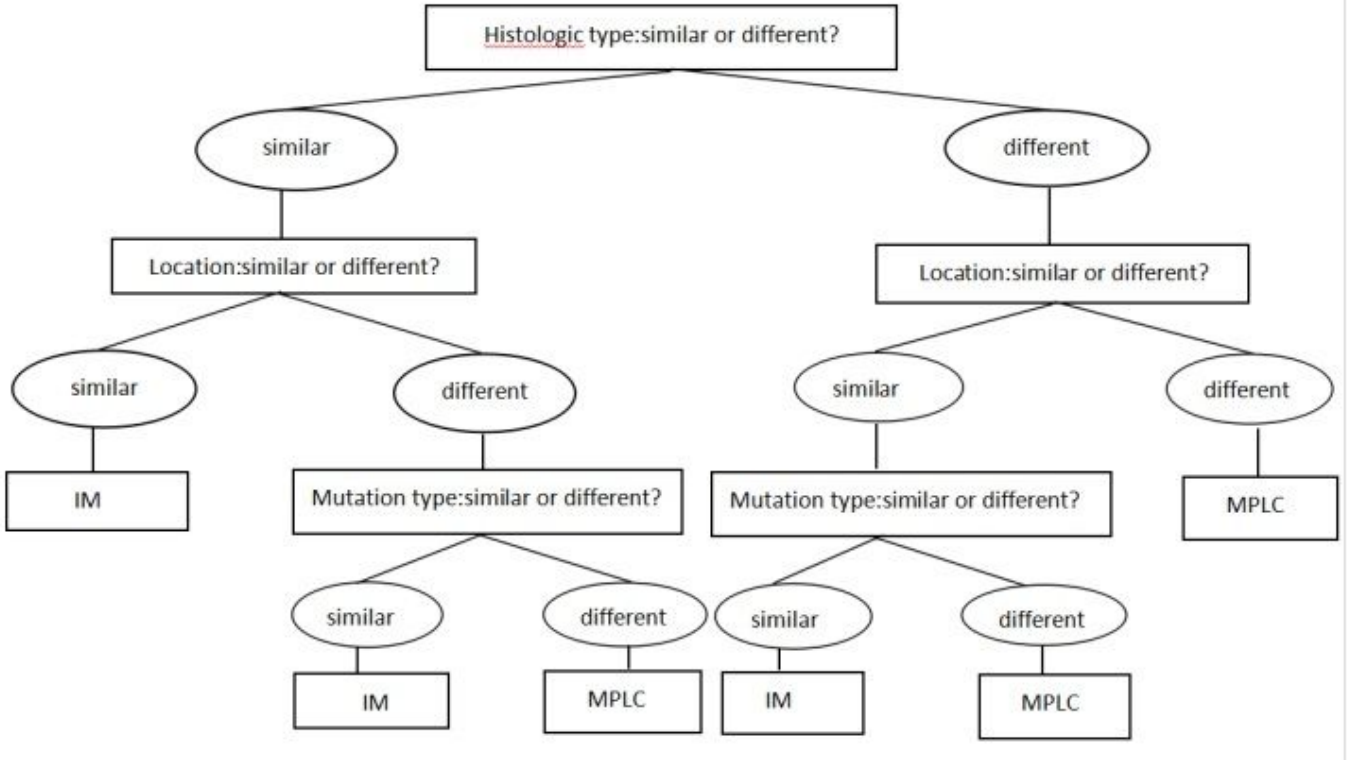

Figure 4

Figure 4