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Clinical and molecular pathological characteristics of 371 cases of malignant pulmonary nodules

LU Xiaomin*, WANG Xiao, ZHENG Haiwen, SUN Yi, ZHU Jiping

**Department of Respiratory and Critical Care Medicine, Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu 21009, China*

Corresponding author: ZHU Jiping, E-mail : zzz-one@126.com

Abstract: Objective To summarize the clinical and molecular pathological characteristics of malignant pulmonary nodules and improve understanding of the disease. **Methods** The study focuses on patients who underwent surgical resection of small pulmonary nodules in Thoracic Surgery of Jiangsu Province Hospital of Chinese Medicine from March 2018 to December 2021 and were pathologically confirmed to have malignant specimens. Retrospective analysis of clinical data and molecular pathological features of surgical specimens was conducted**. Results** A total of 371 patients were included, including 39 cases of carcinoma in situ(CIS), 164 cases of microinvasive adenocarcinoma(MIA) and 168 cases of invasive adenocarcinoma(IA); 69.8% of females, 30.2% of males, with a majority of females.87.6% of patients did not smoke; The upper lobe of the right lung had the most malignant nodules (37.5%), followed by the lower lobe of the left lung (18.6%). In comparion among different pathological types, the ages of CIS, MIA and IA were (54.1±10.9), (52.2±12.6) and (59.1±9.9) years (F=16.05, P<0.01), the smoking rates were 10.3%, 3.7% and 21.4% (χ^2=24.47, P<0.01), the median tumor diameter was 0.55, 0.60 and 0.80 cm (H=76.13, P<0.01), respectively,with IA all being the highest, and the differences were statistically significant. The mutation frequency of exons 19 and 21 of epidermal growth factor receptor (EGFR), and tumor suppressor protein p53 (TP53) in IA was higher than those in CIS and MIA (P<0.05), while the mutation frequency of human epidermal growth factor receptor 2 (HER2, 20ins), mitogen-activated protein kinase (MAP2K1) and serine/threonine protein kinase (BRAF) was lower than those in CIS and MIA, and the differences were statistically significant (P<0.05). **Conclusions** Patients with malignant pulmonary nodules may have the following characteristics: more common in women, non-smoking, located in the upper lobe of the right lung. There were differences in age, smoking rate, tumor diameter and driving gene mutation frequency among the three pathological types of malignant nodules.

Keywords: Small pulmonary nodules, malignant; Carcinoma in situ; Microinvasive adenocarcinoma; Invasive adenocarcinoma; Molecular pathological feature

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Lung cancer is a common malignant tumor in clinical practice, which seriously affects human health. Timely and accurate determination of the nature of pulmonary nodules and multi-disciplinary comprehensive treatment with surgery are of positive significance for improving the prognosis of pulmonary malignant tumors. In recent years, with the popularity of physical examination, the detection rate of pulmonary nodules has increased significantly.

1 Objects and Methods

1.1 Study objects

A total of 371 patients who underwent surgical resection of small pulmonary nodules in Cardiothoracic Surgery Department of Jiangsu Province Hospital of Chinese Medicine from March 2018 to December 2021 were retrospectively selected as the study objects.

Inclusion criteria:

(1) The diameter of the surgical specimen was less than 3 cm, and the pathological examination showed it was lung cancer.

(2) The patient has complete clinical information, has undergone molecular pathological testing for lung cancer, and the data is complete.

Exclusion criteria: metastatic or recurrent lesions.

1.2 Survey Methods

The clinical data was collected from the inpatient records database, including gender, age, smoking status, lung nodule site, pathological type of lung nodule, tumor diameter of lung nodule, and mutated gene detection.

Ethical review This study adopts retrospective study method to analyze the clinicopathological data in the patient's inpatient medical records, which did not cause harm to the human body nor involve the privacy rights or commercial interests of patients' sensitive personal information. With reference to the *Note on Exemption from Ethical Review* of Nanjing University of Chinese Medicine, this study qualified for ethical review exemptions.

1.3 Pathological and genetic testing

Pathological examination of surgical specimens was performed in the Department of Pathology of Jiangsu Province Hospital of Chinese Medicine. Pathology was performed using HE staining method, and diagnostic results were issued after independently read and agreed upon by two pathologists. Genetic mutation testing of the specimens was carried out by Nanjing Geneseeq. All 371 nodules underwent genetic mutation testing, including epidermal growth factor receptor (EGFR, including exons 18,19,20,21), Kirsten mouse sarcoma virus oncogene (KRAS), mesenchymal epithelial transition factor (MET), anaplastic lymphoma kinase (ALK), rearranged during transfection (RET), serine/threonine kinase (BRAF), mitogen activated protein kinase (MAP2K1), human epidermal growth factor receptor 2 (HER2), oncogenic factor 1 receptor tyrosine kinase (ROS1) and tumor suppressor protein p53 (TP53). The test results were reported to the Department of Pathology, and the diagnostic results were issued after review by the molecular pathologist of Nanjing University of Chinese Medicine.

1.4 Statistical Methods

IBM SPSS 26.0 was used for statistical testing. The measurement data conforming to normal distribution were expressed as mean±*SD*. One-way ANOVA was used for multiple groups of measurement data conforming to normal distribution and homogeneity of variance. Turky method was used for two comparisons. Welch analysis of variance was used for data with inconsistent variance, and Games-Howell test was used for pairwise comparison.

Measurement data that did not conform to normal distribution were expressed as M (Q_1, Q_3), Kruskal-Wallis H test was used for multiple groups, and Wilcoxon's signed-rank test was used for multiple comparisons.

The statistical data were represented by case (%). The gender of the patient, the location of pulmonary nodules and the distribution difference between smokers and non-smokers were measured by Chi-square goodness-of-fit test. The statistical data among the three pathological types were compared by chi-square test with R × C contingency tables and its segmentation. Test standard α=0.05.

2 Results

2.1 Demographic characteristics of patients with malignant pulmonary nodules

Among the 371 patients, 69.8% were female and 30.2% were male. Gender distribution did not conform to the equal proportion distribution (χ^2=58.245, P<0.01). The age of female patients was (55.1±12.1) years, and age of male patients was (57.5±10.6) years. There was no significant difference in age between males and females (t=1.82, P=0.07). There were 201 cases (54.2%) aged 55 years and above, and 170 cases (45.8%) aged below 55 years. The difference of age between patients with preinvasive carcinoma, microinvasive adenocarcinoma and invasive adenocarcinoma was statistically significant (F=16.05, P<0.01). There was no statistical difference in the age of patients with preinvasive carcinoma and microinvasive adenocarcinoma (P=0.34), but the age of patients with invasive adenocarcinoma was higher than that of patients with preinvasive carcinoma and microinvasive adenocarcinoma (P<0.05). **Table 1**.

Tab.1 Clinical characteristics of 371 patients with malignant pulmonary nodules

Item	Total	Preinvasive carcinoma	Microinvasive adenocarcinoma	Invasive adenocarcinoma	$\chi^2/F/H$ value	*P* value
Number of cases	371	39	164	168		
Gender (case)					15.90	<0.01
Female	259	24	132	103		
Male	112	15	32	65		
Age (years, $\bar{x}$ ±s)	55.5±11.7	54.1±10.9[a]	52.2±12.6[b]	59.1±9.9	16.05	<0.01
Smoking (case)					24.31	<0.01
Never	325	35	158	132		
Previously/Currently	46	4	6	36		
Surgical site (case)					12.25	0.14
Right upper lobe	139	12	65	62		
Right Middle lobe	37	4	21	12		
Right lower lobe	68	11	23	34		
Left upper lobe	58	8	28	22		
Left lower lobe	69	4	27	38		
Tumor diameter [cm, *M* (Q_1, Q_3)]	0.70(0.50,0.90)	0.55(0.40,0.60)[b]	0.60(0.40,0.70)[b]	0.80(0.62,1.00)	76.13	<0.01

2.2 Pathological types of malignant nodules

Carcinoma in situ accounted for 10.5%, microinvasive adenocarcinoma 44.2%, invasive adenocarcinoma 45.3%, mainly microinvasive adenocarcinoma and invasive adenocarcinoma.

2.3 Smoking status of patients with malignant pulmonary nodules

Among 371 patients with malignant pulmonary nodules, 46 (12.4%) were smokers or former smokers, and 325 (87.6%) were non-smokers. The smoking distribution did not conform to the equal proportion distribution (χ^2=209.814, P<0.01). Among the three pathological types, the smoking rate of invasive adenocarcinoma patients was the highest, and the difference was statistically significant (χ^2=24.31, P<0.01). See Table 1.

2.4 Location of malignant nodules

Malignant nodules in the upper lobe of the right lung were the most common in 139 cases (37.5%). The second was the left inferior lobe of lung, 69 cases (18.6%), the site distribution was not consistent with the equal proportion distribution (χ^2=79.660, P<0.01). There was no significant difference in lesion sites among the three pathological types (χ^2=12.25, P=0.140). See Table 1.

2.5 Tumor diameter

There was significant difference in tumor diameter among the three pathological types of malignant nodules (H=76.13, P<0.01). The diameter of invasive adenocarcinoma was the largest (P<0.01), and there was no significant difference between in situ and microinvasive adenocarcinoma (P>0.05). See Table 1.

2.6 Mutation frequency of driver gene and TP53

Table 2 shows that the mutation frequency of HER2 (20ins), MAP2K1, BRAF, TP53 and EGFRexon21 among the three pathological types of malignant nodules had significant differences (P<0.05). Pair-to-pair comparison showed that there was no difference in mutation frequency between carcinoma in situ and microinvasive adenocarcinoma (P>0.05). EGFRexon19 in invasive adenocarcinoma was significantly higher than that in preinvasive carcinoma. Compared with carcinoma in situ and microinvasive adenocarcinoma, the mutation frequencies of EGFR (exon19, exon21) and TP53 in invasive adenocarcinoma were significantly increased, while the mutation frequencies of HER2 (20ins), MAP2K1 and BRAF were significantly decreased (P<0.05). In addition, HER2 (20ins) mutations were mainly HER2Y772_A775dup, accounting for 79.2% (38/48). BRAF mutation, exon 15 missense mutation V600E mutation in 3 cases, accounting for 10.0% (3/30).

Tab.2 Frequency and mutation rates of driving gene mutations in 371 cases of

malignant pulmonary nodules

Gene	Total mutation rate (%)	Gene mutations of different pathological types [case (%)]			χ^2 value	*P* value
		Preinvasive carcinoma (*n*=39)	Microinvasive adenocarcinoma (*n*=164)	Invasive adenocarcinoma (*n*=168)		
EGFR						
exon18	2.2	2(5.1)	2(1.2)	4(2.4)	2.59	0.23
exon19	18.9	3(7.7)[a]	26(15.9)	41(24.4)	7.52	0.02
exon20	4.6	3(7.7)	6(3.7)	8(4.8)	1.48	0.51
exon21	31.0	5(12.8)[b]	37(22.6)[b]	73(43.5)	23.67	<0.01
KRAS	7.3	1(2.6)	16(9.5)	10(6.0)	3.21	0.20
MET	2.4	2(5.1)	4(2.4)	3(1.8)	1.82	0.37
RET	1.3	0	3(1.8)	2(1.2)	0.45	0.82
ALK	2.7	1(2.6)	4(2.4)	5(3.0)	0.27	0.96
ROS1	0.5	0	0	2(1.2)	1.99	0.60
HER2(ex20ins)	12.9	6(15.4)[a]	36(22.0)[b]	6(3.8)	25.12	<0.01
MAP2K1	5.9	5(12.8)[a]	15(9.1)[b]	2(1.2)	14.72	<0.01
BRAF	8.1	6(15.4)[a]	21(12.8)[b]	3(1.8)	18.92	<0.01
TP53	9.2	1(5.1)[a]	5(3.0)[b]	28(16.7)	20.32	<0.01

3 Discussion

With the wide application of chest CT examination, especially low-dose spiral CT for screening of early lung cancer in high-risk population, the discovery of lung nodules is becoming more and more common [1]. The high-density shadows lesions less than 3 cm in diameter found by chest CT are called "pulmonary nodules". Pulmonary nodules can be divided into ground glass nodules, solid nodules and mixed ground glass nodules according to the imaging findings. 95% of lung nodules are benign, and the high-density imaging is caused by inflammation, granuloma, or small lymph nodes [2]. The incidence of malignant nodules is low. Based on two large cohort data, the Pan-Canadian Early Detection of Lung Cancer Study (PanCan) and the British Columbia Cancer Agency (BCCA), the incidence of malignant nodules is about 5.5% and 3.7% [3]. Timely detection of pulmonary nodules and identification of their good and evil properties can be timely treated in the early stage of lung cancer, and excessive follow-up of benign pulmonary nodules can be reduced, thus optimizing medical costs [4]. Although more advanced imaging techniques such as chest MR [5-6] and 18F-deoxyglucose positron emission computed tomography (FDG PET-CT) [7-9] are also expected to be used to improve the accuracy of benign and malignant identification, many studies have shown that these techniques are not superior to chest CT at present. The chest CT image features of pulmonary nodules were combined with independent predictors such as age, race, smoking status, growth rate, and underlying lung disease to build a prediction model, which improved the accuracy of differential diagnosis [10-11]. The application of artificial intelligence (AI) in chest CT diagnosis has reduced the work intensity of radiologists and improved the accuracy [12-14]. In this study, 371 patients with malignant nodules were observed in terms of age, gender, smoking status and other characteristics, and it was found that the mutation frequency of certain driver genes was different in different pathological stages of malignant nodules. These characteristics are helpful to accurately distinguish benign and malignant pulmonary nodules, and also helpful to discuss the occurrence, development and evolution of lung cancer.

The pathological types of malignant pulmonary nodules are mainly adenocarcinoma, which is a progressive process from in-situ carcinoma to microinvasive adenocarcinoma and invasive adenocarcinoma [15]. Previous studies have shown that older age is associated with the occurrence of malignant pulmonary nodules [3,16]. We found that the age of invasive adenocarcinoma patients was higher than that of orthotopic and microinvasive adenocarcinoma patients, but there was no difference in the mean age of patients with orthotopic and microinvasive adenocarcinoma patients. The older the patients, the higher the malignant degree of malignant nodules. Roy et al. [17] showed that 208 of the 243 cases of ground glass nodules suspected to be malignant were adenocarcinoma, and multivariate analysis showed that females were the risk factor (OR=4.47, *P*=0.002). Some researchers believe that this phenomenon is related to genetic susceptibility and frequent indoor smoke exposure, and point out that current guidelines do not pay enough attention to women as an independent risk factor [18]. The author observed that the gender distribution of malignant nodules was unbalanced, with more females. Roy et al. [17] also found

that Asian people had a higher incidence of malignant nodules. Among 243 cases of ground glass nodules suspected to be malignant, 100% of Asian women and 86% of Asian men were diagnosed with adenocarcinoma, while 84% of non-Asian women and 77% of non-Asian men were diagnosed in the same study. Asian women appear to be more likely to develop malignant lung nodules. Studies have shown that smoking or long-term smoking is associated with the occurrence of malignant pulmonary nodules [19]. However, a systematic review and analysis of the prediction models of benign and malignant pulmonary nodules found that about 50% of the prediction models did not include smoking status as an independent predictor, because most malignant pulmonary nodules are adenocarcinoma, and the occurrence of adenocarcinoma has a low correlation with smoking [10]. The proportion of smoking patients in 371 cases of malignant nodules observed by the author is not high. Therefore, whether smokers are more likely to develop malignant pulmonary nodules remains uncertain, but it should be noted that among the three pathological types, patients with infiltrating adenocarcinoma have the highest smoking rate. The location of malignant nodules is not evenly distributed. BCCA validation dataset analysis showed that the probability of malignant nodules in the upper lobe of the right lung was the highest (1.4%), followed by the lower lobe of the left lung (1.3%) [3]. According to the PanCan dataset, the probability of malignant nodules appearing in the left upper lobe of lung was the highest (1.9%), followed by the right lower lobe (1.7%) [3]. The results of this study were similar to the results of BCCA data set analysis, and the most malignant nodules were distributed in the upper lobe of the right lung.

There was no difference in the frequency of driver mutations between the three pathological types of malignant pulmonary nodules except exon EGFR19/21, BRAF, HER2 (ex20ins), MAP2K1 and TP53. HER2 mutations are present in 2% to 3% of lung adenocarcinoma patients. According to a report by The Cancer Genome Atlas (TCGA), 4% of patients with non-small cell lung cancer (NSCLC) have HER2 mutations [20], of which 90% are HER2 (ex20ins), with A775_G776insYVMA being the most common. Subramanian, Brazel et al. [21-22] showed that HER2 (ex20ins) could continuously activate HER2 kinase, leading to cancer. Therefore, they are classified as driving carcinogenic mutations. HER2 (ex20ins) is associated with women and non-smokers. The study of Nagasaka et al. [23] showed that among 134 tumor specimens with HER2 mutation, HER2 (ex20ins) was the most common, accounting for 69%. Similarly, Tan et al. [24] in the second-generation sequencing of 1 252 lung adenocarcinoma specimens from East Asian patients, the HER2 mutation rate was 3.1%, while the exon 20 insertion mutation accounted for 2.7%. The most common HER2 (ex20ins) is HER2Y772_A775dup at 75%, followed by HER2G776delinsVC at 13%.

Chinese scholars observed 91 lung cancer specimens with HER2 mutation from patients in northeast China, and found that the HER2 mutation in lung cancer mostly occurred in adenocarcinoma, and most of them were stage III and stage IV. There were 14 mutation sub-types, the most common of which were HER2 copy number change (41.76%) and HER2 (ex20ins) (36.26%) [25]. However, it has been reported that the frequency of insertion mutation of ERBB2 in Brazilian NSCLC is very low, and the frequency of HER2 (ex20ins) in 722 NSCLC cases is only 0.8% (6/722), and all of them are adenocarcinomas [26]. The HER2 (ex20ins) mutation rate of malignant pulmonary nodules in this study was different from that reported in the literature [24-25], and the mutation type was mainly $HER2^{Y772\_A775dup}$, which was close to that reported in the literature. [24]

Mitogen-activated protein kinase (MAPK) pathway is an important signaling pathway, which plays an important role in cell survival and proliferation. RAS upstream of the pathway is activated to produce a cascade reaction of RAF-MEK-ERK. There are three types of RAF, namely ARAF, BRAF and CRAF, and their downstream signaling pathways composed of MEK1/2 and ERK1/2 kinases. Genetic alterations in this pathway are most prevalent in human cancers, including many hotspot mutations, such as BRAFV600E [27]. The mutated BRAF protein has elevated kinase activity, leading to infinite cell proliferation and development into cancer [28]. There are different reports on the mutation frequency of BRAF in NSCLC. Roviello et al. [29] reported that the incidence of BRAF gene mutation in NSCLC is relatively low, ranging from 0 to 3%. Riudavets et al. [30] reported that the mutation rate of BRAF in NSCLC was 4%, and Leonetti et al. [31] reported that the mutation rate was 1.5% -3.5%. In 371 cases, BRAF and TP53 were significantly higher in preinvasive carcinoma and microinvasive adenocarcinomas than in invasive adenocarcinomas, and the mutation rate of BRAF was higher than that reported in the literature. Zhang et al. [32] found that EGFR mutation was the most common driving change in in-situ carcinoma, microinvasive adenocarcinoma and invasive adenocarcinoma, while TP53 was found in microinvasive adenocarcinoma and invasive adenocarcinoma, but not in preinvasive carcinoma. Mutations in EGFR, ERBB2, NRAS, and BRAF are believed to be early genomic events during carcinogenesis, while mutations in TP53 and genes associated with cell migration, gap junction, and metastasis may be late events associated with tumor progression. Therefore, TP53 mutation may lead to further increase of tumor aggressiveness.

This study is a small sample of surgical samples of malignant pulmonary nodules. The population and timing of surgery were selected, which may increase the selectivity bias of the study. In addition, the data reported by pathology and molecular pathology in clinical cases are not comprehensive and precise enough. The sample size and comprehensive collection and analysis of molecular biological detection data need to be expanded to improve the accuracy of observation results.

Conflict of Interest None

Reference

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·论　著·

恶性肺小结节 371 例的临床和分子病理学特征

陆晓旻[1]，　王晓[1]，　镇海文[2]，　孙怡[3]，　朱际平[1]

1. 南京中医药大学附属医院 江苏省中医院呼吸与危重症医学科，江苏 南京 210029；

2. 南京中医药大学附属医院 江苏省中医院心胸外科，江苏 南京 210029；

3. 南京中医药大学附属医院 江苏省中医院病理科，江苏 南京 210029

摘要：目的　总结恶性肺小结节的临床和分子病理学特征，提高对该疾病的认识。**方法**　以 2018 年 3 月至 2021 年 12 月于江苏省中医院心胸外科行肺小结节手术切除治疗且病理证实为恶性的患者为研究对象。回顾性分析其临床资料和手术标本分子病理学特征。**结果**　共纳入患者 371 例，原位癌 39 例，微浸润腺癌 164 例，浸润腺癌 168 例；女性 69.8%，男性 30.2%，女性占多数；87.6%患者不吸烟；右肺上叶的恶性结节最多（37.5%），其次为左肺下叶（18.6%）。不同病理类型比较，原位癌、微浸润腺癌和浸润腺癌患者的年龄分别为（54.1±10.9）、（52.2±12.6）和（59.1±9.9）岁（$F=16.05$，$P<0.01$），吸烟率分别为 10.3%、3.7%和 21.4%（$\chi^2=24.31$，$P<0.01$），瘤体直径中位值分别为 0.55、0.60、0.80 cm（$H=76.13$，$P<0.01$），均以浸润腺癌最高，差异均有统计学意义。浸润腺癌表皮生长因子受体（EGFR）的 21 外显子、肿瘤抑制蛋白 p53（TP53）的突变频率高于原位癌和微浸润腺癌（$P<0.05$），而人类表皮生长因子受体 2（HER2）的外显子 20ins、丝裂原活化蛋白激酶（MAP2K1）和丝氨酸/苏氨酸蛋白激酶（BRAF）的突变频率低于原位癌和微浸润腺癌，差异有统计学意义（$P<0.05$）。**结论**　具有恶性肺结节的患者，可能有下列特性：女性、不吸烟、位于右肺上叶多见。三种病理类型的恶性结节中，年龄、吸烟率、瘤体直径和驱动基因突变频率存在差异。

关键词： 肺小结节，恶性；原位癌；微浸润腺癌；浸润腺癌；分子病理学特征

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Clinical and molecular pathological characteristics of 371 cases of malignant pulmonary nodules

LU Xiaomin*, WANG Xiao, ZHEN Haiwen, SUN Yi, ZHU Jiping

** Department of Respiratory and Critical Care Medicine, Jiangsu Province Hospital of Chinese Medicine, Affiliated Hospital of Nanjing University of Chinese Medicine, Nanjing, Jiangsu 21009, China*

Corresponding author: ZHU Jiping, E-mail: zzz-one@126.com

Abstract: Objective　To summarize the clinical and molecular pathological characteristics of malignant pulmonary nodules and improve understanding of the disease. **Methods**　Patients who underwent surgical resection of small pulmonary nodules in Cardiothoracic Surgery of Jiangsu Province Hospital of Chinese Medicine from March 2018 to December 2021 and were pathologically confirmed to be malignant were taken as the research objects. Retrospective analysis of clinical data and molecular pathological features of surgical specimens was conducted. **Results**　A total of 371 patients were enrolled, including 39 cases of carcinoma in situ (CIS), 164 cases of microinvasive adenocarcinoma (MIA) and 168 cases were invasive adenocarcinoma (IA); 69.8% were females, 30.2% were males, with a majority of females. 87.6% of patients did not smoke; The upper lobe of the right lung had the most malignant nodules (37.5%), followed by the lower lobe of the left lung (18.6%). In comparison among different pathological types, the ages of CIS, MIA and IA were (54.1±10.9), (52.2±12.6) and (59.1±9.9) years ($F=16.05$, $P<0.01$), the smoking rates were 10.3%, 3.7% and 21.4% ($\chi^2=24.31$, $P<0.01$), the median tumor diameter was 0.55, 0.60 and 0.80 cm ($H=76.13$,

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通信作者： 朱际平，E-mail: zzz-one@163.com

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$P<0.01$), respectively, with IA all being the highest, and the differences were statistically significant. The mutation frequency of exons 21 of epidermal growth factor receptor (EGFR), and tumor suppressor protein p53 (TP53) in IA were higher than those in CIS and MIA ($P<0.05$), while the mutation frequency of human epidermal growth factor receptor 2 (HER2) exon 20ins, mitogen-activated protein kinase (MAP2K1) and serine/threonine protein kinase (BRAF) were lower than those in CIS and MIA, and the differences were statistically significant ($P<0.05$). **Conclusion** Patients with malignant pulmonary nodules may have the following characteristics: more common in women, non-smoking, located in the upper lobe of the right lung. There are differences in age, smoking rate, tumor diameter and driving gene mutation frequency among the three pathological types of malignant nodules.

Keywords: Small pulmonary nodules, malignant; Carcinoma in situ; Microinvasive adenocarcinoma; Invasive adenocarcinoma; Molecular pathological feature

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肺癌是临床上常见的恶性肿瘤,严重影响人类的健康。及时和准确判断肺结节的性质并通过以手术为主的多学科综合治疗,对于改善肺恶性肿瘤的预后具有积极意义。近年随着健康体检的普及,肺部结节的检出率显著增加。本研究对 371 例恶性肺结节患者的临床资料进行回顾性分析,总结其临床和手术标本的分子病理学特征。

1　对象与方法

1.1　*研究对象*　回顾性选取 2018 年 3 月至 2021 年 12 月在江苏省中医院心胸外科行肺结节手术切除治疗的 371 例患者为研究对象。纳入标准:(1) 手术标本病灶直径小于 3 cm,病理检查为肺癌。(2) 患者临床信息完整,接受肺癌分子病理检测,且数据完整。排除标准:手术病灶为转移性或复发性病灶。

1.2　*调查方法*　从住院病历资料库获取资料,收集数据包括患者性别、年龄、吸烟状况、肺结节部位、肺结节的病理类型、肺结节瘤体的直径、突变基因检测情况。本研究采用回顾性研究方法,分析患者住院病历中的临床病理资料,不对人体造成伤害,不涉及患者个人敏感信息的隐私权或商业利益,参照南京中医药大学《关于免除伦理审查的说明》,符合临床研究免除伦理审查的情况。

1.3　*病理和基因检测*　手术标本的病理学检查在该院病理科进行。采用 HE 染色,由两位病理科医生独立完成读片并达成一致意见后出具诊断结果。标本的基因突变检测由南京世和基因生物技术实施,371 例结节均进行了表皮生长因子受体[EGFR,包括外显子(exon)18,19,20,21]、Kirsten 鼠肉瘤病毒癌基因(KRAS)、间充质-上皮转化因子(MET)、间变性淋巴瘤激酶(ALK)、转导重排基因(RET)、丝氨酸/苏氨酸蛋白激酶(BRAF)、丝裂原活化蛋白激酶(MAP2K1)、人类表皮生长因子受体 2(HER2)、肉瘤致癌因子 1 受体酪氨酸激酶(ROS1)和肿瘤抑制蛋白 p53(TP53)基因的突变检测,检测结果反馈至病理科,经本院分子病理诊断医师审核后发出诊断结果。

1.4　*统计学方法*　统计软件采用 IBM SPSS.26。计量资料符合正态分布用 $\bar{x}\pm s$ 表述,符合正态分布、方差齐性的多组计量资料采用单因素方差分析,两两比较采用 Turky 法,如方差不齐,采用 Welch 方差分析,两两比较采用 Games-Howell 检验。不符合正态分布的计量资料以 $M(Q_1,Q_3)$ 表述,多组间采用 Kruskal-Wallis H 检验,多重比较采用 Wilcoxon 秩和检验。计数资料采用例或例(%)表述,患者性别、肺结节所在部位及吸烟者与非吸烟者的分布差异采用 χ^2 拟合优度等比例检验;三种病理类型间计数资料的比较采用 R×C 表 χ^2 检验及其分割法。$P<0.05$ 为差异有统计学意义。

2　结　果

2.1　*恶性肺结节患者的人口学特征*　入组 371 例患者,女性患者占多数,为 69.8%,男性为 30.2%,性别分布不符合等比例分布($\chi^2=58.245$, $P<0.01$)。女性患者年龄(55.1±12.1)岁,男性年龄(57.5±10.6)岁,男性与女性年龄差异无统计学意义($t=1.82$, $P=0.07$)。55 岁及以上 201 例(54.2%),55 岁以下 170 例(45.8%)。原位癌、微浸润腺癌和浸润腺癌患者的年龄分别为(54.1±10.9)、(52.2±12.6)和(59.1±9.9)岁,三者间差异有统计学意义($F=16.05$, $P<0.01$);两两比较,原位癌和微浸润腺癌患者的年龄差异无统计学意义($P=0.34$),浸润性腺癌患者年龄分别大于原位癌、微浸润腺癌患者,差异均有统计学意义($P<0.05$)。见表 1。

2.2　*恶性结节的病理类型*　原位癌占 10.5%,微浸润腺癌占 44.2%,浸润腺癌占 45.3%,以微浸润腺癌、浸润腺癌为多。

2.3　恶性肺结节患者的吸烟状况　371 例恶性肺结节中正在吸烟或曾经吸烟的患者 46 例(12.4%),不吸烟者占大多数,为 325 例(87.6%),吸烟分布不符合等比例分布(χ^2=209.814, P<0.01)。三种病理类型患者吸烟率差异有统计学意义(χ^2=24.31,P<0.01),浸润腺癌患者吸烟率最高。见表 1。

2.4　恶性结节部位　右肺上叶的恶性结节最多,139 例(37.5%);其次为左肺下叶,69 例(18.6%),部位分布不符合等比例分布(χ^2=79.660, P<0.01)。三种病理类型患者病变部位差异无统计学意义(χ^2=12.25, P=0.140)。见表 1。

2.5　瘤体直径　三种病理类型的恶性结节,瘤体直径差异有统计学意义(H=76.13,P<0.01)。两两比较,浸润腺癌瘤体直径最大(P<0.01),原位癌与微浸润腺癌之间差异无统计学意义(P>0.05)。见表 1。

2.6　驱动基因和 TP53 的突变频率　表 2 可见,三种病理类型的恶性结节在 HER2(20ins)、MAP2K1、BRAF、TP53 及 EGFRexon19、21 的突变频率上差异有统计学意义(P<0.05)。两两比较显示,原位癌和微浸润腺癌间突变频率未见差异(P>0.05);与原位癌相比,浸润腺癌的 EGFRexon19 显著增高(P<0.05);与原位癌、微浸润腺癌相比,浸润腺癌的 EGFRexon21、TP53 的突变频率显著增高,而 HER2(20ins)、MAP2K1、BRAF 的突变频率显著降低(P<0.05)。此外,HER2(20ins)突变以 $HER2^{Y772\_A775dup}$ 为主,占 79.2%(38/48)。BRAF 突变,15 外显子错义突变 V600E 突变 3 例,占 10.0%(3/30)。

表 1　371 例恶性肺结节患者的临床特征

Tab. 1　Clinical characteristics of 371 patients with malignant pulmonary nodules

项目	合计	原位癌(n=39)	微浸润腺癌(n=164)	浸润腺癌(n=168)	$\chi^2/F/H$ 值	P 值
性别(例)						
女	259	24	132	103	15.90	<0.01
男	112	15	32	65		
年龄(岁, $\bar{x}\pm s$)	55.5±11.7	54.1±10.9[a]	52.2±12.6[b]	59.1±9.9	16.05	<0.01
吸烟状况(例)						
从不吸烟	325	35	158	132	24.31	<0.01
曾经/正在吸烟	46	4	6	36		
手术部位(例)						
右肺上叶	139	12	65	62		
右肺中叶	37	4	21	12		
右肺下叶	68	11	23	34	12.25	0.14
左肺上叶	58	8	28	22		
左肺下叶	69	4	27	38		
瘤体直径[cm, $M(Q_1,Q_3)$]	0.70 (0.50,0.90)	0.55 (0.40,0.60)[b]	0.60 (0.40,0.70)[b]	0.80 (0.62,1.00)	76.13	<0.01

注:与浸润腺癌比较,[a]P<0.05,[b]P<0.01。

表 2　371 例恶性肺结节驱动基因突变频数和突变率

Tab. 2　Frequency and mutation rates of driving gene mutations in 371 cases of malignant pulmonary nodules

基因	总突变率(%)	不同病理类型的基因突变[例(%)] 原位癌(n=39)	微浸润腺癌(n=164)	浸润腺癌(n=168)	χ^2 值	P 值
EGFR						
exon18	2.2	2(5.1)	2(1.2)	4(2.4)	2.36	0.31
exon19	18.9	3(7.7)[a]	26(15.9)	41(24.4)	7.52	0.02
exon20	4.6	3(7.7)	6(3.7)	8(4.8)	1.20	0.55
exon21	31.0	5(12.8)[b]	37(22.6)[b]	73(43.5)	23.67	<0.01
KRAS	7.3	1(2.6)	16(9.5)	10(6.0)	3.21	0.20
MET	2.4	2(5.1)	4(2.4)	3(1.8)	1.49	0.47
RET	1.3	0	3(1.8)	2(1.2)	0.85	0.63
ALK	2.7	1(2.6)	4(2.4)	5(3.0)	0.09	0.85
ROS1	0.5	0	0	2(1.2)	2.43	0.30
HER2[c]	12.9	6(15.4)[b]	36(22.0)[b]	6(3.8)	25.12	<0.01
MAP2K1	5.9	5(12.8)[b]	15(9.1)[b]	2(1.2)	13.13	<0.01
BRAF	8.1	6(15.4)[b]	21(12.8)[b]	3(1.8)	16.68	<0.01
TP53	9.2	1(5.1)[a]	5(3.0)[b]	28(16.7)	20.77	<0.01

注:与浸润腺癌比较,[a]P<0.05,[b]P<0.01;[c] 为 HER2(20ins)。

3　讨　论

随着胸部 CT 检查的广泛应用,特别是低剂量螺旋 CT 用于高风险人群早期肺癌的筛查,肺结节的发现也越来越常见[1]。胸部 CT 检查发现的直径小于 3 cm 的密度增高的病灶被称为“肺结节”。肺结节根据影像表现可分为磨玻璃结节、实性结节和混杂磨玻璃结节。95%的肺结节是良性的,是由于炎症、肉芽肿或小淋巴结造成的高密度影像[2]。恶性结节的发生率较低,通过对 Pan-Canadian Early Detection of Lung Cancer Study (PanCan)和 British Columbia Cancer Agency (BCCA)两大队列数据的研究发现,恶性结节的发生率约为 5.5%和 3.7%[3]。及时发现肺结节并鉴别其良恶性质,可以在肺癌的早期阶段及时处理,也能减少对良性肺结节的过度随访,从而优化医疗成本的支出[4]。尽管也希望利用胸部 MR[5-6]、^{18}F-脱氧葡萄糖正电子发射计算机断层显像/计算机断层成像(FDG PET-CT)[7-9]等更先进的影像技术提高良恶性鉴别的准确度,但诸多研究表明,目前这些技术并不优于胸部 CT。将肺结节的胸部 CT 影像特征和患者的年龄、种族、吸烟状况和生长速率、基础肺疾病等独立预测因子共同构建预测模型,提高了鉴别诊断的准确性[10-11]。在胸部 CT 诊断中采用人工智能技术(AI),减轻了放射科医生的工作强度,也提高了准确度[12-14]。本研究观察 371 例恶性结节患者的年龄、性别、吸烟状况等特征,发现不同病理阶段的恶性结节,其某些驱动基因突变频率不相同。这些特征有助于准确鉴别肺结节的良恶性,对

探讨肺癌的发生和发展演化也有帮助。

恶性肺结节的病理类型主要为腺癌,由原位癌向微浸润腺癌和浸润腺癌的渐进过程[15]。既往的研究表明,年龄较大与恶性肺结节发生相关[3,16]。笔者观察发现,浸润腺癌患者的年龄大于原位癌和微浸润腺癌,而原位癌和微浸润腺癌患者平均年龄没有差异,患者年龄越大,恶性结节的恶性程度也高。Roy 等[17]的研究表明,在怀疑是恶性的 243 例磨玻璃结节中 208 例为腺癌,多因素分析显示,女性是危险因素(OR=4.47,P=0.002)。有研究者认为,这种现象与基因易感性和频繁的室内油烟暴露有关,并且指出目前的指南对于女性作为独立危险因素的重视程度不够[18]。笔者观察发现,恶性结节的性别分布不均衡,女性多。Roy 等[17]的研究还发现,亚裔人群恶性结节的发生率更高,在怀疑是恶性的 243 例磨玻璃结节中,100% 亚裔女性被确诊为腺癌,亚裔男性为 86%,而同一研究中非亚裔女性为 84%,非亚裔男性为 77%。亚洲女性似乎更容易出现恶性肺结节。有研究表明吸烟或曾经长期吸烟与恶性肺结节的发生有关[19]。但对肺结节良恶性的预测模型进行系统综述分析发现,约 50% 的预测模型并未将吸烟状态列为独立预测因子,因为恶性肺结节多数为腺癌,而腺癌的发生和吸烟的相关性比较低[10]。笔者观察的 371 例恶性结节中吸烟患者所占的比例不高。因此吸烟者是否更易发生恶性肺结节仍不确定,但是应注意三种病理类型中,浸润腺癌患者吸烟率最高。恶性结节的部位分布不均衡。BCCA 验证数据集分析显示右肺上叶的恶性结节的概率最高(1.4%),其次为左肺下叶(1.3%)[3]。而 PanCan 数据集分析左肺上叶恶性结节出现的概率最高(1.9%),其次为右肺下叶(1.7%)[3]。本研究的结果与 BCCA 数据集分析结果相似,恶性结节分布最多的部位在右肺上叶。

恶性肺结节的三种病理类型间,除了 EGFR19、21 外显子,BRAF、HER2(20ins)、MAP2K1 和 TP53 外,其他的驱动基因突变频率未见差异。2%~3% 的肺腺癌患者存在 HER2 突变。Cancer Genome Atlas 的一份报告显示,4% 的非小细胞肺癌(NSCLC)患者存在 HER2 突变[20],这些突变 90% 为 HER2(20ins),其中 A775_G776insYVMA 最为常见。Subramanian、Brazel 等[21-22]研究表明,HER2(20ins)可以使 HER2 激酶持续激活,导致癌症发生。因此,它们被归类为驱动致癌突变。HER2(20ins)与女性和不吸烟者有关。Nagasaka 等[23]的研究显示在 134 例 HER2 突变的肿瘤标本中,HER2(20ins)最为常见,达 69%。同样,Tan 等[24]在 1 252 例来自东亚患者的肺腺癌标本的二代测序中,HER2 突变率为 3.1%,而外显子 20 插入突变占 2.7%。最常见的 HER2(20ins)是 $HER2^{Y772\_A775dup}$ 占 75%,其次是 $HER2^{G776delinsVC}$ 占 13%。中国学者观察了 91 例来自中国东北患者存在 HER2 突变的肺癌标本,发现在肺癌中 HER2 的突变多发生在腺癌,且多为Ⅲ、Ⅳ期,突变类型共有 14 个亚型,最常见的是 HER2 拷贝数改变(41.76%)和 HER2(20ins)(36.26%)[25]。而有研究报道巴西 NSCLC ERBB2 的插入突变发生频率很低,在 722 例的 NSCLC 中 HER2(20ins)的发生频率仅为 0.8%(6/722),且均为腺癌[26]。本研究恶性肺结节 HER2(20ins)突变率与文献报道存在差异[24-25],突变类型以 $HER2^{Y772\_A775dup}$ 为主,与文献报道接近[24]。

MAPK 通路是重要的信号通路,对细胞的生存和增殖有重要的作用。通路上游的 RAS 激活后产生 RAF-MEK-ERK 的级联反应。RAF 有三种类型分别是 ARAF、BRAF 和 CRAF,以及它们的下游 MEK1/2 和 ERK1/2 激酶组成的信号通路。该通路的基因改变在人类癌症中最为普遍,其中包括许多热点突变,如 BRAFV600E[27]。突变的 BRAF 蛋白具有升高的激酶活性,导致细胞无限增殖,发展成癌[28]。BRAF 在 NSCLC 中的突变频率报道不一,Roviello 等[29]报道 NSCLC 中 BRAF 基因突变的发生率较低,约为 0~3%。Riudavets 等[30]报道在 NSCLC 中 BRAF 的突变率为 4%,Leonetti 等[31]报道突变率为 1.5%~3.5%。本组 371 例的恶性结节中,BRAF 和 TP53 在原位癌和微浸润腺癌中明显高于浸润腺癌,BRAF 突变率高于文献报道。Zhang 等[32]的研究发现,EGFR 突变是原位癌、微浸润腺癌和浸润腺癌中最常见的驱动改变,而 TP53 在微浸润腺癌和浸润腺癌中被发现,但在原位癌中未被发现;因此认为,EGFR、ERBB2、NRAS 和 BRAF 的突变是癌变过程中的早期基因组事件,而 TP53 以及与细胞迁移、间隙连接和转移相关的基因突变,可能是与肿瘤进展相关的晚期事件。因此 TP53 突变会导致肿瘤侵袭性进一步增高。

本研究是对恶性肺结节的手术样本的小样本观察。接受手术的人群和时机是经过选择的,可能增加研究的选择性偏倚。此外,临床病例中病理学和分子病理报告的数据不够全面和精确。有待扩大样本量,以及全面的分子生物学检测数据的搜集和分析,以提高观察结果的准确性。

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