Original Article
A DNA quantitative analysis of lung cancer cells with different pathological types from bronchial brush specimens and its clinical significance

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Abstract: Objective: To explore the significance of a DNA quantitative analysis of lung cancer cells with different pathological types taken from bronchial brush specimens and its relationship with the clinicopathological features. Methods: 903 bronchial brush cytological specimens taken in the Cytology Department of the Fourth Hospital of Hebei Medical University were collected from March 2017 to December 2019 and divided into three groups: the squamous carcinoma (SC) group, the adenocarcinoma (AC) group, and the small-cell carcinoma (SCC) group. The maximum DNA index (DI) value, the percentage of cells with a DI larger than 2.5, the mean DI, and the peaks of the aneuploid cells of the three groups were compared. A single factor analysis was used to analyze the relationship between the DNA content, aneuploidy, and the clinicopathological features of the patients who had surgery. Results: The peaks of the aneuploid cells in the SC group, the AC group, and the SCC group had no significant differences (*P*=0.57). The maximum DI, the percentage of cells with a DI larger than 2.5, and the mean DI of the three groups showed statistically significant differences (*P*<0.001). The clinicopathological features of the AC patients and the SC patients, such as gender, age, tumor type, the maximum tumor diameter, clinical stage, vascular invasion, nerve invasion, pleural invasion, tracheal spread, and lymph node metastasis were not independent factors that influence the DNA content and have no statistical significance (*P* > 0.05). Conclusion: The reason why the DNA content of small-cell lung cancer is lower than SC and AC remains to be further studied.

Keywords: DNA content, squamous cell carcinoma, adenocarcinoma, small-cell carcinoma, clinicopathological characteristics

Introduction

Lung cancer is the malignant tumor with the highest morbidity and mortality, ranking first in China and even in the world [1, 2]. Most patients are diagnosed too late, so early diagnosis is key to treating the disease. A method of diagnosing of lung cancer early, bronchoscopy has the advantages of causing little trauma, convenient specimen preparation, and high repeatability [3, 4]. Using the brush specimens obtained from bronchoscopies, a better diagnostic rate can be obtained. As an ancillary method of cytological diagnosis, DNA quantitative analysis is widely used for early diagnoses because of its repeatability and sensitivity [5, 6]. However, there are few studies on the relationship between the DNA content and the biological behavior of different pathological types

of lung cancer. Therefore, in this study, the correlation of the DNA content with the different pathological types of lung cancer cells and its clinical significance were investigated.

Materials and methods

Materials

903 cases of bronchial brush smears in from the Cytology Department of the Fourth Hospital of Hebei Medical University were collected from March 2017 to December 2019. Each participant filled out an informed consent form, and this study was approved by the hospital ethics committee. Their clinicopathological information was collected. Among the patients, 143 underwent surgical operations and had surgical specimens taken.

Methods

Using bronchoscopy, all the patients underwent a brush biopsy after lesions were found. The brushed cells were smeared on six slides, four of which were used for routine cytological diagnosis and classification using HE staining and immunocytochemistry. The remaining two were dyed with Feulgen staining for an automatic DNA quantitative analysis. The histological specimens obtained through bronchoscopy bite biopsies and percutaneous transthoracic biopsies under CT guidance were sent to the histopathology department for examination.

Diagnosis

The cytological and histopathological diagnoses were made by a team of two experienced cytologists and two experienced pathologists. The cytological and histological specimens with matching results were included, and those with conflicting results were excluded.

DNA quantitative analysis

LD DNA image cytometry (LD DNA-ICM, Landing Med Tech, Wuhan) was used for the DNA quantitative analysis. The DNA-ICM included a Wave NP370D2 server, an Olympus BX41 microscope, a ueye M2240 camera, and an automatic control platform. The DNA-ICM was used to determine the DNA content by measuring the integrated optical density (IOD) of the stained nucleus DNA, using normal lymphocytes on the same slide as the control. The DNA content was expressed using the DNA Index (DI=DNA IOD of the tested cell/DNA IOD average of normal cell). The DNA content was usually measured in c (content). 1c was half of the DNA content of normal G0/G1 cells. When the tested cell was in the G0/G1 phase, its IOD value was very close to the average IOD value of normal cells, so its DNA index was 1, namely 2c. When the DNA content of the tested cells formed peaks between 2c~4c, 4c~8c and 8c~16c; or when there were more than 3 cells with a DI larger than 2.5 (5c), tumors should be highly suspected.

Aneuploid cell peaks included: ① type I no peak, which shows aneuploid cells of DI > 2.5 are scattered everywhere; ② type II single peak, which can appear in different positions, DI mostly between 1-2; ③ type III double peaks, which can appear in a variety of tumors, and the DI of the second peak is often twice that of the first peak; ④ type IV multi-peaks, and the histogram shows different heights like Manhattan houses, and the appearance of multiple peaks indicates that the chromosome structure of the tumor is very unstable (**Figure 1**). The maximum DI, the percentage of cells with DI > 2.5, mean DI (mean DI of the first 20 representative tumor cells) and the peaks of the aneuploid cells were used as the statistical indicators for the DNA quantitative analysis. The number of cells on the slides used for the DNA quantitative analysis should be no less than 2000.

Clinicopathological features

Age, gender, maximum tumor diameter, tumor type, lymph node metastasis, and other relevant information about the patients whose surgical specimens were collected.

Statistical methods

SPSS 26.0 software was used for the statistical analysis. The DNA quantitative analysis indexes were tested using Kruskal-Wallis and Bonferroni tests, and the relationships between the DNA content and the clinicopathological features were tested using Mann-Whitney U tests. $P < 0.05$ was considered a statistically significant difference.

Results

Clinicopathological features of the patients

A total of 903 patients were enrolled, with a median age of 63, and ranging in age from 31 to 87. Among them, 682 were men and 221 were women. The diagnoses determined that 337 cases were SC, 268 were AC, and 298 were SCC. Among the 143 patients with surgical specimens, 121 were male and 22 were female, ranging in age from 39 to 78, with a median age of 62. The diagnoses indicated that 95 cases were SC and 48 were AC.
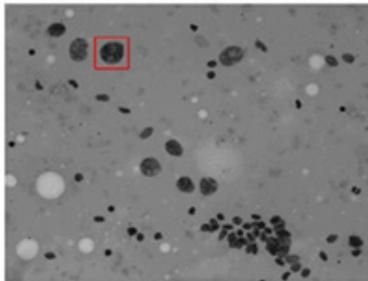
Results of the DNA quantitative analysis

There were no significant differences in the SC, AC and SCC aneuploid cell peaks (P=0.057). But the maximum DI value, the percentage of cells with a DI > 2.5, and the mean DI value in
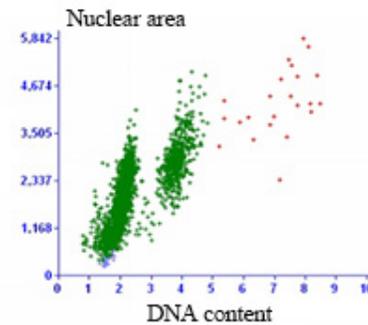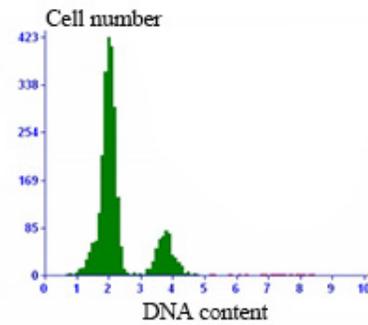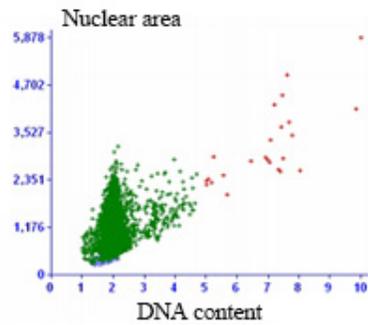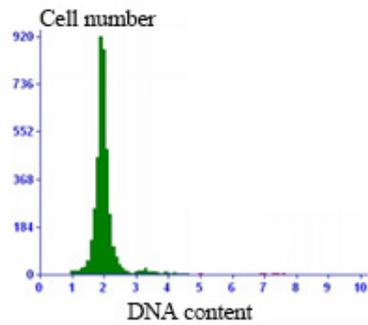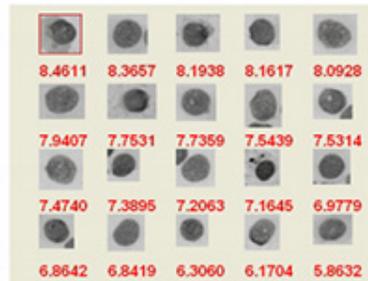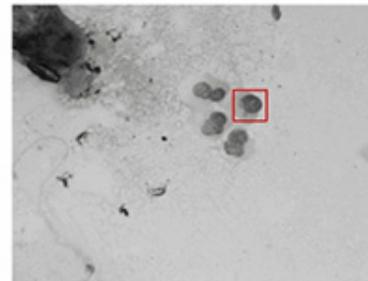
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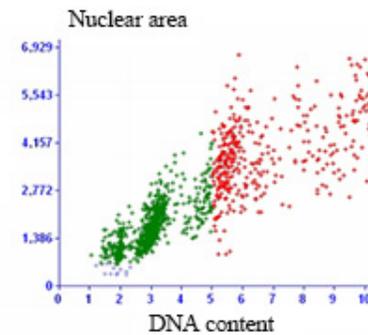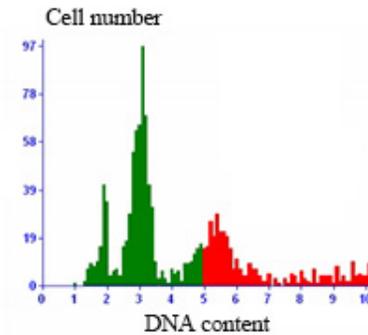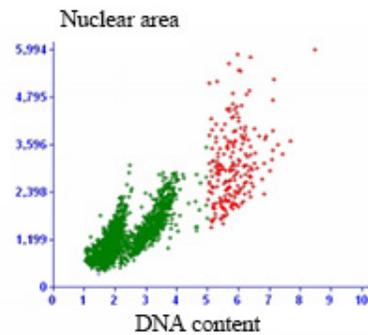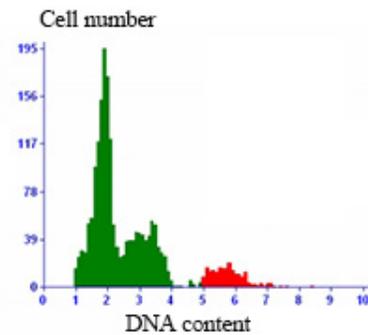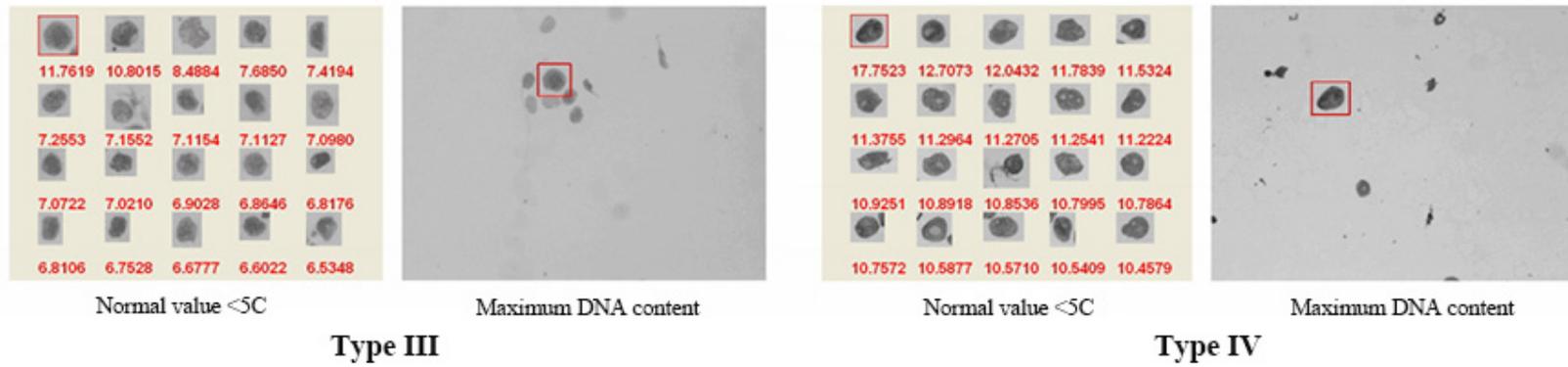
Figure 1. DNA quantitative analysis.

Table 1. The DNA analysis results of SC, AC, and SCC

Pathology type	the maximum DI				percentage of cells with DI larger than 2.5			
	Median	Average rank	statistical quantity	p	Median	Average rank	statistical quantity	p
SC	5.35	478.98	33.779	<0.001	1.78	484.75	40.492	<0.001
AC	5.42	479.14			2.20	497.91		
SCC	4.81	380.90			0.94	373.67		

Pathology type	mean DI				the peaks of aneuploid cells		
	median	Average rank	statistical quantity	p	Average rank	statistical quantity	p
SC	3.50	459.75	29.74	<0.001	197.60	1.04	0.60
AC	3.64	509.93			210.85		
SCC	3.48	391.14			202.39		

Table 2. The DNA analysis results of SC, AC ,and SCC using pairwise comparisons

Pathology type	the maximum DI		percentage of cells with DI larger than 2.5		Content mean DI	
	statistical quantity	p	statistical quantity	p	statistical quantity	p
SC-SCC	98.082	<0.001	111.079	<0.001	40.290	0.156
AC-SCC	116.239	<0.001	124.232	<0.001	118.508	<0.001
SC-AC	-18.157	1.000	-13.153	1	-78.219	<0.001

the three groups showed a significant difference by comparison (**Table 1**). The maximum DI and, the percentage of SC and AC cells with a DI > 2.5 were higher than the corresponding SCC values ($P<0.001$), and the above two indicators in SC and AC showed no significant differences ($P=1$). The average DI value of AC was higher than the average DI value of SC and SCC ($P<0.001$), and the average DI value of SC was slightly higher than the average DI value of SCC, but the differences was not statistically significant ($P=0.156$) (**Table 2**).

The relationship between the DNA quantitative analysis and the clinicopathological features

The patients' clinicopathological features, such as gender, age, maximum tumor diameter, type of tumor, pleural invasion, vascular invasion, nerve invasion, lymph node metastasis, and stage were not independent influencing factors of the DNA content ($P > 0.05$) (**Table 3**).

Discussion

Lung cancer is one of the most common malignant tumors in China. The incidence of lung cancer (35/100,000) in China is comparable to that in US, but the five-year survival in China is lower, and the death rate is 1.4 times that of the United States [7, 8]. The reason is that with most patients in China the disease is found in the advanced stages, so early diagnosis is the key to treating the disease. In the clinic, CT, x-rays, and other imaging examinations play important roles in detecting pulmonary diseases. However, for the diagnosis of lung tumors, the pathological results are the gold standard. Bronchoscopy, a common method of testing for lung cancer, has a high diagnosis rate for central lung cancer and peripheral lung cancer invading bronchi. The lesion can be directly observed, and cyto- and histopathological specimens can be obtained using brush and bite biopsies. Bronchial brush biopsies can brush the lesion site with a wider range, so they can acquire diseased specimens more easily and successfully than bite biopsies [9-11].

DNA, a carrier of genetic information, plays an important role in cell growth, differentiation and reproduction [12]. When a malignant tumor occurs, the tumor genes undergo point mutation, fusion and amplification, and as the tumor progresses, the DNA content increases significantly. An automatic DNA image analysis system is used to measure the DNA content of the detected cells. This technique overcomes

Table 3. A single factor analysis of the DNA content, aneuploidy, and clinicopathological features

clinicopathological features	NO.	the maximum DI		percentage of cells with DI larger than 2.5		Content mean DI		the peaks of aneuploid cells	
		Z	P	Z	p	Z	p	Z	p
Gender		-1.007	0.314	-0.490	0.624	-0.257	0.797	-0.952	0.341
men	121								
women	22								
Age		-0.413	0.680	-0.668	0.504	-1.630	0.103	-1.191	0.234
<62	66								
≥62	77								
Tumor type		-0.325	0.745	-0.543	0.587	-0.675	0.499	-0.910	0.363
SC	95								
AC	48								
Tumor diameter		-0.591	0.555	-2.104	0.035	-0.340	0.734	-0.608	0.543
<4 cm	62								
≥4 cm	81								
Stage		4.050	0.132	1.736	0.420	0.722	0.697	2.750	0.253
I	44								
II	44								
III	55								
Vascular Invasion		-0.597	0.550	-0.360	0.719	-0.457	0.648	-0.051	0.960
yes	8								
no	135								
Nerve invasion		-0.884	0.377	-0.744	0.457	-1.993	0.46	-2.040	0.41
yes	7								
no	136								
Pleura invasion		-0.627	0.531	-0.468	0.640	-0.181	0.857	-0.199	0.842
yes	25								
no	118								
Tracheal spread		-0.600	0.549	-0.258	0.797	-0.050	0.960	-1.387	0.165
yes	30								
no	113								
Lymph node metastasis		-0.854	0.393	-0.968	0.333	-0.213	0.832	-0.105	0.916
yes	66								
no	77								

missed diagnoses and overdiagnosis that occur due to fatigue and humans' strong subjectivity, so it is more objective. It has been widely used in the auxiliary diagnosis of cytology because of its objectivity, repeatability, and high sensitivity [13-19]. Many studies have shown that the DNA content level and the number of aneuploid cells are related to tumor occurrence, proliferation, invasion, and prognosis [20-35]. The poorer the differentiation of the tumor, the higher the DNA content and the more aneuploid cells [36]. However, there are few studies comparing the DNA content and the aneuploid cells among different pathological types of the same organ tumor.

Our study was designed to determine whether the DNA content and the aneuploid cells were different in different pathological types of lung cancer, and whether they can predict the biological behavior of lung cancer.

As early as 1982, Hirsch et al. [37] proposed that small-cell lung cancer could spread widely at the early stage. Yokota et al. [38] believed that small-cell lung cancer progressed faster than lung AC and SC. Byrd et al. [39] analyzed the imaging manifestations of common lung tumors and found that hilar lymph nodes of small-cell lung cancer tend to be early and heavily involved compared with other types of

cancer. Later, Chen et al. [40] also mentioned that small-cell lung cancer is the most invasive subtype, with rapid growth, high proliferation, early transmission, and a wide spread, and even brain metastasis can occur in more than half of the patients. Pfeifer et al. [41] and Dai et al. [42] found that P53 gene mutations were more common in small-cell lung cancer and suggested a poor prognosis. According to previous studies and the observations of clinical cases, SCC is more malignant. Our study compared the DNA content of lung SC, AC, and SCC and found that the maximum DI and the mean DI of lung AC and SC were higher than the corresponding SCC values. Therefore, DNA content cannot be used separately to evaluate the malignancy of different pathological types of lung cancer, especially small cell cancer. Kogan's research [43] showed that the higher the DNA content, the more aneuploid cells and the less differentiated the lung squamous carcinoma and adenocarcinoma, but they found that smallcell lung carcinoma, a special type of tumor unlike other types of carcinoma, is characterized by a low, sometimes diploid DNA content which does not correlate with its malignancy, and this finding is similar to those of our study.

Our study also investigated the correlation of DNA content and aneuploidy with the clinicopathological features of lung cancer, like gender, age, tumor type, maximum tumor diameter, clinical stage, vascular invasion, nerve invasion, pleural invasion, tracheal spread, and lymph node metastasis, and found no statistically significant differences, so perhaps the patients we studied who had surgery were too small a cohort, and our research question needs further study.

Conclusion

(1) Although the DNA content is different in different pathological types of lung cancer, it cannot be used alone to compare the levels of malignancy among the different pathological types of lung cancer, especially small-cell lung cancer. (2) The reason why the DNA content of small-cell lung cancer is lower than SC and AC remains to be further studied. (3) The correlation between DNA content and aneuploidy and the clinicopathological features of lung cancer was not determined in this study, so further research is needed.

Disclosure of conflict of interest

None.

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