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COMMENTARY

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New method for discrimination of multiple primary lung cancer and metastatic lung cancer

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Lung cancer is currently the leading cause of death of cancer patients worldwide.[1] The rate of morbidity resulting from multiple primary lung cancer (MPLC) has increased during these years. Patients with multiple primary lung cancers can be divided into synchronous or metachronous according to the time of appearance of the lesions.[1,2] Therefore, the correct clinical identification of MPLC or intrapulmonary metastasis of lung cancer is beneficial to individualized treatment. The clinicopathological criteria for the diagnosis of multiple primary lung cancers were first proposed by Martini and Melamed in 1975.[3,4] In this study, we used the comet assay to further differentiate MPLC from intrapulmonary metastases.

Of the 70 consecutive patients with primary lung cancer who had undergone a surgical resection between January 2000 and July 2022 at the Department of Thoracic Surgery, West China Hospital, Sichuan University, Chengdu, China, 30 patients were diagnosed with MPLCs according to the Martini and Melamed criteria. Of these patients, 18 patients had synchronous MPLCs and 12 had metachronous MPLCs of different histological type. In addition, 40 patients diagnosed with intrapulmonary metastasis during the same period were included for comparison. The clinicopathological data were obtained from a retrospective chart review (Table 1). The experiments were approved by the West China Hospital Ethics Committee (no. 202133) and all participating patients provided informed consent.

Blood samples were collected in two green top heparinized vacutainer tubes (BD Diagnostics). These were light protected, and immediately transported at room temperature to the molecular laboratory. We performed the comet assay under alkaline conditions as described by Singh et al.[5] with minor modifications.

The DNA damage rate of peripheral blood mononuclear cells in the pulmonary metastatic carcinoma (PMC) group was 6.43% (180/2800), which was significantly higher than 3.76% (113/2998) of the multiple primary lung cancer group ($X^2 = 18.983$, $p = 0.000$). Meanwhile, the DNA damage tail length, tail moment and olive tail moment (OTM) of patients in the pulmonary metastatic carcinoma group (44.21 ± 12.35 μm, 4.41 ± 1.36, 4.68 ± 1.32) was significantly higher than in the multiple primary lung cancer group (32.16 ± 7.45 μm, 3.12 ± 1.21, 3.33 ± 1.09) ($p = 0.000$) (Tables 2 and 3).

Ideally, genetic markers for clonality analysis should be independent and highly frequent somatic mutations that occur early and remain unchanged throughout the carcinogenesis process. There is substantial evidence that there are individual differences in the ability of endogenous and exogenous carcinogens to DNA damage, which contributes to cancer risk.[6] The DNA repair capacity of patients with lung cancer is lower than that of normal controls, which further increases the risk of lung cancer.[7,8]

In the present study, genetic measures were obtained with the comet assay. This assay was utilized to measure constitutive genetic instability or baseline damage. Briefly, by this method, one can determine the constitutive or unrepaired DNA damage represented by shorter DNA strands that migrate faster in the electrophoretic field and resemble the tail of a comet. The DNA damage rate of peripheral blood mononuclear cell in the pulmonary metastatic

TABLE 1 Clinicopathological data of all patients

	Synchronous multiple lung cancers	Metachronous multiple lung cancers	Intrapulmonary metastasis
Characteristics	No. of patients	No. of patients	No. of patients
Patients in study	18	12	40
Sex			
Male	16	10	16
Female	2	2	24
Age, years (mean [range])	70.5 [43–84]	68.1 [46–82]	66.4 [54–79]
Site of tumor			
LUL + LLL	8	3	9
RUL + RML	2	1	2
RUL + RLL	3	4	18
RML + RLL	2	1	1
LUL + RLL	1	2	4
LUL + RUL	2	1	6
Histological type			
Adenocarcinoma	-	-	26
Squamous cell carcinoma	-	-	14
Follow-up			
Alive	18	11	27
Dead	0	1	13

Abbreviations: LLL, left lower lobe; LUL, left upper lobe; RLL, right lower lobe; RML, right middle lobe; RUL, right upper lobe.

TABLE 2 The differences in peripheral nucleated cell DNA damage rate in two groups

Group	No. of patients	Total cell	Cell damage	Damage rate (%)	X^2	*p*-value
MPLC	30	2998	113	3.76	18.983	0.000
PMC	40	2800	180	6.43	-	-

Abbreviations: MPLC, multiple primary lung cancer; PMC, pulmonary metastatic carcinoma.

TABLE 3 The differences of peripheral nucleated cell DNA damage level in two groups

	Tail length(um)		Tail moment		Olive moment	
Group	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value
MPLC	32.16 ± 7.45	0.000	3.12 ± 1.21	0.000	3.33 ± 1.09	0.000
PMC	44.21 ± 12.35	-	4.41 ± 1.36	-	4.68 ± 1.32	-

Abbreviations: MPLC, multiple primary lung cancer; PMC, pulmonary metastatic carcinoma.

carcinoma group was 6.29%, which was significantly higher than 3.77% of the MPLC group. At the same time, the DNA damage tail length, tail moment and olive moment of patients in the pulmonary metastatic carcinoma group was significantly higher than the multiple primary lung cancer group. We found that the results in four cases of intrapulmonary metastasis were significantly higher than the other patients in this group.

Obviously, in clinical practice, the identification of MPLC and intrapulmonary metastases is of great significance for the disease staging and management, as well as prognosis of patient. Alternative methods of implementing genetic and/or molecular testing criteria could provide more efficient information for definitive diagnosis of these suspected cases. Elevated levels of damaged DNA are associated with the development of multiple NSCLC tumors in NSCLC patients.[9] However, due to the relatively small number of cases analyzed in this study, larger cohort studies are needed to confirm these results in the future.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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