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Clinical and Pathological Research Status of Multiple Pulmonary Nodules

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Abstract

With the changes in disease spectrum and the popularization of screening of low-dose spiral CT (CT) in the chest, more and more pulmonary nodules have been detected, most of which are bipulmonary multiple nodules. The existence of multiple pulmonary nodules means that it may be a pathological state of benign and malignant co-existence. The origin and evolution of pulmonary nodules in different histopathological states have a great impact on the choice of treatment methods. In recent years, the rise of immunotherapy has brought a breakthrough in the treatment of refractory lung cancer. However, some patients are still ineffective in immunotherapy, which may be related to the immune microenvironment where nodules are proportioned in different components in different pathological states. This review article mainly predicts the development process of nodules by analyzing the origin of multiple pulmonary nodules and the immune microenvironment of nodules in different pathological conditions, so as to provide guidance for clinical treatment.

Keywords

Lung Nodules, Originate, Pathology, Immune Microenvironment

1. Introduction

According to the latest global cancer statistics, lung cancer is the second most common cancer after breast cancer and has the highest mortality rate, accounting for 25% - 30% of all cancer deaths, with a 5-year survival rate of only about 20% [1] [2]. With the improvement of medical imaging technology, lung cancer

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screening plans, and monitoring of previously treated cancer patients, the incidence of lung nodules has been increasing, and the detection rate has gradually increased. Previously, even small nodules could be accurately detected, and most of the detected patients showed multiple scattered pulmonary nodules in both lungs. Multiple scattered pulmonary nodules mostly indicate early-stage multiple lung cancer, especially nodules with EGFR, KTM2C, KRAS, and TP53 mutation patterns. KTM2C may be a new driver gene associated with KRAS mutation in the early development of lung adenocarcinoma [3]. However, not all lung nodules are equivalent to lung cancer, and these nodules cover every stage from benign to atypical to malignant processes. Benign pulmonary nodules can be divided into congenital and acquired, with inflammatory granulomas being the most common, accounting for about 80% of all benign nodules [4]; the pathological histological types of malignant pulmonary nodules are mainly various histological subtypes of lung adenocarcinoma, and about 80% of lung cancer histological types are adenocarcinoma [5], including the entire development process of atypical adenomatous hyperplasia (AAH), adenocarcinoma in situ (AIS), minimally invasive adenocarcinoma (MIA), and invasive adenocarcinoma (IAC) [6]. The diagnosis and treatment of pulmonary nodules require comprehensive analysis and comprehensive diagnosis from multiple aspects such as pathological morphology, molecular pathology, imaging and clinical. This article predicts the progression of lung nodules and guides treatment by comprehensively analyzing the clinical and pathological characteristics of multiple pulmonary nodules and changes in the immune microenvironment.

2. Overview of Multiple Pulmonary Nodules

Pulmonary nodules refer to isolated or multiple pulmonary shadows with a diameter of ≤3 cm on imaging. The occurrence of pulmonary nodules is closely related to smoking. Whether it is a smoking population or a second-hand smoke population, coupled with the increasingly severe atmospheric pollution [7] [8], the age of onset has gradually transitioned from aging to younger ages. The clinical symptoms of pulmonary nodules are not obvious, and most of them occur in females [9]. Isolated nodules can be distributed in any segment of the lung and appear as solitary nodules; while multiple pulmonary nodules refer to the presence of two or more diffuse nodules in the lung at the same time, which can be manifested as solid or sub-solid density shadows on imaging, the latter of which includes pure ground-glass shadows and partially solid shadows [10]. Pure ground-glass nodules are usually caused by benign reasons (such as infection), while if the nodules have solid components (or partially solid), the nodules may be malignant; in addition, slowly growing malignant tumors may present as in situ adenocarcinoma with pure ground-glass density [11]. However, whether it is isolated or multiple pulmonary nodules, the assessment and management of benign and malignant risks are definitely universal issues that need to be addressed, as they have a great impact on clinical treatment strategies and unne-

cessary operations for non-cancer patients. Smoking, aging, and a history of tumors are risk factors that can increase the probability of malignant tumors.

In the detection and diagnosis of pulmonary nodules, chest CT plays an important role. The morphology, size, and distribution of nodules usually help distinguish the tumor causes. The CT characteristics of malignant tumors are generally manifested as spiculated edges and tumor vascular signs, rapid growth, and the presence of solid components with a ground-glass appearance [12]. Most benign nodules have clear borders, satellite nodules around the lesion, slow growth and proliferation, relatively small volume, and generally disappear or even completely calcify in about 2 - 3 years [13]. The nodules have a higher benign rate with smaller volume and fewer solid components, as compared by density and size [14] [15].

In addition, a study evaluated the performance characteristics of comprehensive blood proteomics classification based on protein and clinical parameters to distinguish benign and malignant nodules. It was found that two proteins, LG3BP and C163A, which are independently associated with lung cancer and cancer-related inflammation, can predict low-risk nodules. In particular, LG3BP overexpression is related to lung cancer pathways and related transcription factors [16]. Similarly, biomarkers also play an important role in identifying the population at highest risk. To demonstrate the rationality in clinical utility research, the accuracy of the biomarker, understanding of the potential benefits of true results, and the harm of false results are estimated. In an early lung cancer detection test, seven autoantibodies were measured by enzyme-linked immunosorbent assay (ELISA), and each autoantibody had individual specificity for the following tumor-related antigens: p53, NY-ESO-1, CAGE, GBU4-5, HuD, MAGE A4, and SOX2. The results showed that the seven autoantibodies in early lung cancer testing showed high specificity and moderate sensitivity when detecting lung cancer after two years, and the latter may be related to the decrease in autoantibody production and detection induced by tumor-induced immune response suppression [17]. In patients with pulmonary nodules, early lung testing enhances the positive predictive ability of nodule-based risk assessment for detecting lung cancer. The high specificity of early lung testing can be combined with CT to show high sensitivity, ensuring a high detection rate of early lung cancer cases.

In summary, distinguishing between benign and malignant multiple nodules is the result of a comprehensive evaluation, and when necessary, can be clarified through positron emission tomography-computed tomography (PET-CT) detection and tissue biopsy. PET-CT, as a qualitative diagnostic tool, can have a sensitivity and specificity of about 80% for lung cancer, while tissue biopsy can provide a more definitive diagnosis.

3. Origin of Malignant Multiple Pulmonary Nodules

The lung cancer-lymph node metastasis (TNM) classification system developed

by the International Association for the Study of Lung Cancer (IASLC) shows that lung cancer with multiple lesions in imaging studies can be divided into four categories: secondary primary lung cancer, isolated tumor nodules (lung metastasis), multiple ground-glass opacities, and pneumonia-type pulmonary adenocarcinoma [18] [19]. Malignant multiple pulmonary nodules generally have three sources: 1) Multiple primary cancers; 2) Primary lung cancer with lung metastasis; 3) Other malignant tumors metastasized to the lungs. Multiple primary lung cancer (MPLC) refers to two or more primary malignant tumors that occur simultaneously or successively in the lungs of the same patient without N2/N3 lymph node or systemic metastasis. A key issue that needs to be addressed urgently at present is the differential diagnosis between MPLC and intrapulmonary metastasis (IPM), especially if the tumors exhibit the same histological type. A Recent case report study shows that a 45-year-old woman developed seven lung lesions, including primary tumor and metastatic lesions, histopathological examination of surgical specimens showed that (except the left upper and lower lobe mass due to small size), an invasive adenocarcinoma (LPA) and five microinvasive adenocarcinoma, lesions with a high degree of histological similarity [20]. Distinguishing between MPLC and IPM has a significant impact on tumor staging and treatment selection. Classifying multiple lung nodules as different primary or intrapulmonary metastases may affect treatment selection and determine whether patients receive surgical treatment instead of chemotherapy and/or radiotherapy. Some standards have been proposed to address this diagnostic issue, but there is still a lack of a clear consensus.

Pathological evaluation of multiple pulmonary nodules plays an important role in distinguishing between multiple primary tumors and intrapulmonary metastases. In multifocal lung cancer, pathologists should use comprehensive histological subtypes, immunophenotypes, and molecular research results to distinguish synchronous (and asynchronous) primary tumors and intrapulmonary metastases. Histological subtype analysis should be performed first in multifocal lung cancer to collect relevant evidence supporting or opposing a single tumor origin. Different patterns of biomarkers and the absence of lymph node or systemic metastasis favor tumors unrelated to biology (single primary tumor). Conversely, the same pattern of biomarkers or the presence of significant lymph node or systemic metastasis provides relative evidence for biologically related tumors (intrapulmonary metastases). The most useful morphological features for distinguishing multiple primary tumors from metastatic tumors are the primary tumor type, primary histological pattern, acinar formation, nuclear pleomorphism, cell and nucleolus size, and mitotic rate [21].

It is also difficult to determine whether synchronous multiple ground-glass nodules (SMGGN) in imaging studies are pulmonary metastases of the same primary lung cancer or multiple primary lung cancers (MPLC) from different sources. SMGGN usually has the same histological type, even if they have different growth patterns [22]. According to the traditional definition, MPLC with

the same histological results must be evaluated based on the following criteria: 1) Histological origin is from the primary site of cancer; 2) No involvement of lymph nodes in the conventional lymph node metastasis pathway; 3) No extrathoracic metastasis [23] [24]. In addition, in patients with synchronous multiple ground-glass nodules (SMGGN) that are difficult to distinguish whether they are primary tumors or pulmonary metastatic lesions, the necessity of PET/CT and enhanced brain MRI examinations are still needed.

In summary, distinguishing the source of multiple lung cancer nodules is crucial for the clinical management of lung cancer patients, as it affects staging, prognosis, and treatment selection [25]. In fact, compared to intrapulmonary metastases, multiple primary lung cancers have lower staging and better prognosis, with more surgical options for the former and requiring active chemotherapy or targeted therapy for the latter [25] [26] [27]. Currently, clinical features, imaging, and pathological characteristics are mainly used to comprehensively determine and distinguish MPLC and pulmonary metastasis. However, it is difficult to distinguish between the two if the pathological type and imaging characteristics are similar. Recently, advances in mutation research have provided a molecular basis for identifying MPLC and intrapulmonary metastases. A reseach queried 4119 NSCLCs, which > 1 surgically removed lung cancer patients, analyzed by 341 - 468 gene MSK-IMPACT NGS test. The results support the overall accuracy of comprehensive histological assessment in identifying tumor relationships in most NSCLC, while also highlighting its limitations in approximately one-fifth of cases. As a powerful complementary tool for routine histological diagnosis, comprehensive NGS can explicitly describe the clonal relationships between NSCLC [28].

Next-generation sequencing (NGS) technology can be used for whole-genome sequencing, whole-exome sequencing, or targeted gene sequencing of surgical specimens to analyze whether multiple ground-glass nodules (GGN) have the same origin [29]. This is necessary for comprehensive management of multiple GGN patients. Multiple studies have shown a high concordance rate of gene mutations between primary lung tumors and matched metastases [30]. However, this information can not be used as definitive evidence, as the same mutation may occur by chance in different tumors (and has been found in tumors with different morphologies), driver mutations may also occur in normal appearing lung tissues, germ line mutations (e.g. EGFR) may be lethal, and tumors may exhibit genetic heterogeneity, with a discordance rate of up to 45% between primary and metastatic tumors [31] [32] [33] [34]. Tumors with essentially identical molecular results are considered to originate from a clone (metastasis), while those with inconsistent results are considered independent primary tumors. In different series, differences between the clinical and molecular classifications of initially suspected multiple primary lung cancer cases range from 18% to 30% [35] [36]. Therefore, identifying the same mutation in two tumors does not necessarily indicate a clonal relationship. Such results should always be correlated

with tumor morphology and imaging results. In summary, molecular testing for oncogenic mutations in advanced lung adenocarcinoma has become standard testing methods, and are becoming more and more important tools.. Therefore, in staging multiple lung tumors, information about the mutation status of genes can often be combined with histological evaluation of the tumor. The NGS platform has been implemented in many clinical laboratories, which offer simultaneous detection of gene mutations, copy number changes, and gene rearrangements, providing a better lineage identification method.

4. The Immune Microenvironment of Multiple Pulmonary Nodules

The lung is a major site of immune regulation and can generate powerful and highly regulated immune responses to protect the host from pathogen infections. Epithelial cells, dendritic cells, macrophages, neutrophils, eosinophils, B lymphocytes, T lymphocytes, and many other cells can promote pulmonary immunity. Increasing evidence shows that stromal cells, such as macrophages, T cells, and fibroblasts, exhibit high heterogeneity in both tumor and healthy lung tissue [37] [38]. Like many other cancers, lung cancer is a disease of intracellular heterogeneity caused by multiple factors.

In the tumor microenvironment, the interaction between immune cells and the pathological subtypes of cancer has a significant impact on cancer prognosis and treatment response. Most patients with multiple lung nodules diagnosed as lung adenocarcinoma go through a progression from in situ adenocarcinoma (AIS) to minimally invasive adenocarcinoma (MIA), and finally to invasive adenocarcinoma (IAC). In less invasive pathological subtypes, the percentage of tumor-infiltrating T cells is higher, while in more invasive pathological subtypes, the percentage of macrophages in the tumor tissue is higher, indicating that T cells play a role in inhibiting tumor invasion, while other cells such as macrophages, regulatory T cells, and cancer-associated fibroblasts have the opposite effect [39].

Studies have shown that the compartments of T cell and NK cell are significantly altered in stage I lung adenocarcinoma lesions [39]. $CD8^+$ effector T cells, T cells, and NK cells are enriched in normal tissues, while immature T cells and regulatory T cells are enriched in tumors, indicating that tumors have an inhibitory effect on T cells in their microenvironment. Compared with AIS and MIA, adjacent normal tissues in IAC are enriched with $CD8^+$ effector cells, indicating their potential role in tumor suppression. Similarly, $CD8^+$ effector cells are more enriched in MIA than in AIS, which may be due to the increased neoantigen load leading to higher antigenicity of the tumor in the process of developing from AIS to MIA, attracting more immune cells. In summary, $CD8^+$ effector T cells and NK cells mainly come from normal tissues, while regulatory T cells and immature T cells mainly come from MIA and IAC tumor tissues [39].

B cells can directly reduce tumor invasion, alter pathological status, and

change tumor heterogeneity. It has been reported that there is no significant difference in B cells between cancer and para-cancerous tissues, and the plasma cell subtype in lung adenocarcinoma is an independent negative prognostic factor, possibly one of the main producers of the immune upregulating cytokine IL-35 [40].

Myeloid cells are a diverse group of immune cells that can sense and respond to tissue damage by clearing damaged cells and promoting the recruitment of immune effector cells [41]. Macrophages and dendritic cells play important roles in coordinating immune responses and responding to potential threats. In addition to their innate immune functions, they can also present tumor-associated antigens to T cells [42] [43]. Dendritic cells (DCs) are important accessory cells that play a crucial role in initiating primary immune responses. DCs (mainly conventional type 2 dendritic cells, cDC2) are efficient antigen-presenting cells and have been shown to have a significant impact on clinical outcomes in various cancers [44]. The proportion of cDC2 in normal tissues and cancer tissues is significantly different, with a significantly higher proportion of cDC2 in tumors than in normal tissues. This result suggests that tumor antigens may be presented to effector cells. However, the true functional status of cDC2 in the tumor microenvironment and its role in tumors need further research to confirm. In the respiratory system, mast cell activity participates in maintaining healthy lungs through innate and adaptive immunity against pathogen infections [45]. Mast cells accumulate in lung adenocarcinoma in a vascular-dependent manner. The accumulation of mast cells may be related to the vascular endothelial growth factor released by tumors, and stromal mast cells are associated with angiogenesis and poor outcomes in stage I lung adenocarcinoma [46]. In summary, the myeloid subgroups in AIS are dominated by mast cells, while macrophages dominate in MIA and IAC [39].

By comparing nodules and adjacent normal tissues, it was found that topoisomerase IIα (TOP2A), matrix metalloproteinase 15 (MMP15), and MX dynamin-like GTPase 2 (MX2) are highly expressed in tumor tissues. The TOP2A gene encodes the topoisomerase IIα protein, which is a nuclear enzyme essential for chromosome separation during mitosis. MMPs (matrix metalloproteinases) are a family of zinc and calcium-dependent enzymes that participate in the degradation of various extracellular matrix components. The MX2 gene belongs to the large GTPase family [47]. The expression of TOP2A, MMP15, and MX2 may also be related to the occurrence of adenocarcinoma.

5. Summary and Outlook

Currently, the identification of the origin of pulmonary nodules is still a comprehensive process, including the comprehensive evaluation of clinical imaging, histopathology, and molecular pathology, in order to accurately describe the characteristics of lung cancer and pulmonary metastases. Identifying the origin of pulmonary nodules has a positive significance for the treatment management

of the patient's primary lesion and guiding prognosis. Various immune cells in the tumor immune microenvironment play important roles in the growth, metastasis, and metabolism of cancer cells. During the development from benign to malignant, the proportions of immune cell components in the immune microenvironment of different stages of the disease are also different. The differences discovered have significant implications for the early diagnosis of lung cancer, potential treatment strategies, and improving prognosis. Previous research and clinical guidelines have mostly focused on solitary pulmonary nodules, with very few guidelines for the diagnosis and treatment of multiple pulmonary nodules. Clinical physicians mainly refer to guidelines related to solitary pulmonary nodules to assess the benign and malignant nature of multiple pulmonary nodules. Currently, there is no clear research direction specifically for multiple pulmonary nodules. By integrating the specific immune markers, pathological tissue characteristics and gene mutations of multinodular patients, the origin problem can be more accurately analyzed and provide reliable information for clinical practice. With the rapid development of molecular genetics and scientific technology, future research can focus on the nature and development trends of multiple pulmonary nodules, as well as clinical treatment and prognosis plans.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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