

Possible Factors that Influencing the Extant of Infiltration of Immune Cells in Lung Cance

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Abstract:

Lung cancer has become one of the most common infectious cancers in the world today, causing a large number of deaths every year. Most current research focuses on using PD-1 inhibitors in combination with chemotherapy to treat lung cancer. However, the effect of this treatment plan varies from person to person. It is not effective for patients with low PD-L1 expression, and has the same degree of side effects and poor prognosis. In this article, he systematically introduced the epidemiological knowledge of lung cancer and conducted a comprehensive analysis of the mechanism of tumor cell escape. On this basis, he focused on analyzing the influencing factors of immune cell infiltration (ICI). These influencing factors can provide comprehensive references for subsequent research. In addition, this article conducts a comprehensive comparison of different methods for detecting the degree of infiltration, which can provide a reference for the development of more effective methods in the future.

Keywords: Lung cancer; tumor cell; immune cell; immune cell infiltration.

1. Introduction

With the development of modern medicine, more and more diseases have been found a proper way to be treated. Lung cancer, however, is still a tough problem that human hardly have strategies to deal with. In 2018, according to the WHO Global Health Observatory data repository for age-adjusted prevalence of current smoking, it is said by GLOBOCAN that there are 11.6% of total cancer cases (2.09 million) and 18.4% of total cancer deaths (1.76 million), making it the most common cancer and kill most [1]. Programmed cell death protein/ligand 1 (PD-1/PD-L1) inhibitor mono-therapy and PD-1 inhibitor combined with chemo-therapy are commonly used as first-line treatment for non-small cell lung cancer (NSCLC). Clinical studies [2] have shown that patients with high expression of PD-L1 are more likely to benefit from immunotherapy. However, for patients with low or negative PD-L1 expression, the survival benefit of immunotherapy is limited, and the existing treatment regimens cannot meet the needs of such patients in the real world. Exploring new effective or efficient treatment regimens is critical to further improve the efficacy and prognosis of NSCLC in different populations. As a result, although many methods for treating lung cancer have been developed, it is still an urgent problem to realize the individualization of lung cancer treatment, carry out personalized treatment according to the specific conditions of different patients, improve the early diag-

nosis rate of lung cancer. For the time being, the therapy that strengthens autoimmune cells with drugs to clear the tumor cells has gained popularity. How to increase the extent of infiltration of immune cells in cancer, avoiding the occurrence of immune escaping is a problem in urgent need of solution.

This article will try to explore the potential factors which influencing the degree of infiltration of immune cells in cancer and increase that degree in clinic to relieve the symptoms of patients and even kill related cancer cells. Studying the factors affecting infiltration of immune cells can help relieve the symptoms of patients, providing a possible cure for lung cancer.

2. Research and Treatment of Lung Cancer

2.1 Epidemiological Background and Classification of Lung Cancer

In many countries, lung cancer is the domain cause of cancer-related death. Lung cancer is the second most commonly seen in cancer incidence and rank first for cancer mortality and the age-standardized rate of incidence and mortality were 22.4 and 18.0 per 100,000 globally [3]. Lung cancer is often divided into small cell lung cancer (SCLC) and NSCLC according to the site of onset of lung cancer. Usually it takes about two to five years for NSCLC to develop into metastasis stage (M1), at which stage

the lung cancer may spread directly into nearby tissue or other distant parts of the body via the lymphatic circulation or haematogenous spread. Lung cancer often becomes incurable at M1 stage. For SCLC, however, it only takes 3 months to half a year to develop into M1. Among all lung cancers, NSCLC make up for about 80-85%, including large cell carcinoma, adenocarcinoma and squamous cell carcinoma, etc., with larger cell morphology and relatively slow growth rate. SCLC differs significantly from NSCLC in terms of biological characteristics. SCLC usually expresses high levels of some cytokines and growth factors, such as tumor necrosis factor- α , interleukin-1, interleukin-6, etc., which can promote the growth and invasion of tumor cells [4]. In addition, SCLC is sensitive to chemotherapy and radiotherapy, but is prone to recurrence and metastasis. NSCLC has different biological characteristics, and its cell growth and invasion are regulated by a variety of signaling pathways, such as epidermal growth factor receptor (EGFR), KRAS, PI3K, etc. According to different signaling pathways, a variety of targeted drugs have been applied to the treatment of NSCLC, such as EGFR inhibitors, ALK inhibitors, etc. Besides, the clinical manifestations of SCLC and NSCLC are also different. SCLC often presents respiratory symptoms at an early stage, such as cough, expectoration, shortness of breath, etc., progresses rapidly, and is prone to lymph node metastasis and distant metastasis. NSCLC usually has no obvious symptoms in the early stage. Once symptoms appear, it is often progressed to the middle and late stage. Common symptoms include cough, expectoration, shortness of breath, chest pain, etc.

2.2 Treatment Options and Limitations

Common treatments for early-stage lung cancer include removing the tumor cells by surgical methods, chemotherapy and radiotherapy. It is obvious that the operation can do significant harm to the human body. Radiation and chemotherapy will kill healthy cell when trying to remove cancer cells, accompanied with hair loss, bladder bleeding, nausea, vomiting and decrease in resistance. It often uses radiation to penetrate tissue to apply treatment, which is extremely destructive to human body.

High-throughput next-generation sequencing (NGS) technology [5] has showed its potential to treat cancer. It is sensitive as it not only enables people to test multiple genomic mutations at the same time, but has the ability to detect genomic abnormalities, except some specific situations like point mutations or small insertions or deletions. However, there are some issues with this approach. The most obvious one is the management of large genomic datasets, taking too long to analyze the data correctly. The second is that this method always generates unknown data, which are difficult to interpret. Economic cost is the

third drawback. To address the emergence of acquired resistance in tumor cells, Next generation drugs and combination strategies targeting resistant clones (in the early stage or disease progression) are often used, but emerging toxicity and tolerance are another challenge. To address the heterogeneity of tumors, people now commonly use liquid biopsies and next-generation clinical trials, but lacking robust test development and clinical translation of routine care.

Immune checkpoint inhibitors, combined with other conventional treatments (such as radiotherapy and chemotherapy), have a place in the treatment of NSCLC, which is supported by certain theoretical and clinical data [6]. Moreover, there are some particular molecules on the surface of cancer cells, which have the ability to slow down or completely mask the expression of the signature antigens on the surface of cancer cells, thus avoiding being recognized by immune cells. That is why it always difficult to clear cancer cells depending on patients' own immune system. If the infiltration of immune cells to lung cancer cells can be enhanced, maybe a therapy centered on human immune system, relying less on those harmful treatments, will be discovered. Not only the cost will decrease, but the patients will also get better therapeutic effect.

3. Immune Cells Recognize and Attack Tumor Cells

Tumor cells, as illustrated in Figure 1, exhibit distinct biological properties that allow them to elude immune surveillance, including aberrant cell cycle regulation, unchecked proliferation, and apoptosis, among others. There are numerous methods in which tumor cells can evade the immune system. A common characteristic of the tumor microenvironment (TME) of different types of cancer is hypoxia [7]. Through the following channels, tumor cells in the TME impede immune cell function. First, some molecules, including PD-L1, can suppress immune cells by their expression, which prevents immune cells from attacking tumor cells. Secondly, they have the ability to generate immune-suppressive cells, which inhibit immune system activity. By preventing immune cells from activating, these cells lessen the strength of the immunological response. Additionally, it may be able to achieve immune evasion with the use of modifications in epigenetic traits. Lastly, the TME allows tumor cells to evade the immune system. By interacting with stromal and vascular cells, tumor cells can encourage the growth and spread of tumors. They can also suppress the immune system by secreting substances that depress the immune system.

However, the immune system has a powerful monitoring and clearance function, and can recognize and attack

tumor cells through the synergistic action of a variety of immune cells. Firstly, immune cells recognize antigens on tumor cells through surface receptors, which can be tumor-specific antigens (such as virus-induced tumor antigens) or tumor-related antigens (such as glycolipids, proteins, etc.). Dendritic cells (DCs) are often considered to play the most important role in the immune system. They can take up antigens produced by tumor cells, process them into peptides, and then present them to T cells through MHC molecules. Next, activated T cells regulate immune responses through a variety of cytokines and chemokines. Some T cells (such as cytotoxic T lymphocytes, CTLs) can directly kill tumor cells, and cause tumor cell apoptosis by releasing cytotoxins such as perforin and granzyme. Another part of T cells (such as helper T lymphocytes, Th) can assist B cells to produce antibodies, which can bind to the antigens on the surface of tumor cells, activate the complement system or kill tumor cells through antibody-dependent cell-mediated cytotoxicity (ADCC). In addition, natural killer cells (NK cells) are important anti-tumor effector cells in the non-specific immune system. They can directly identify and kill tumor cells, and play a role in natural immune surveillance. At the same time, immunomodulatory cells (such as regulatory T cells, Treg) play a negative regulatory role in tumor immune response. They can inhibit the activity of CTL and reduce the intensity of immune response.

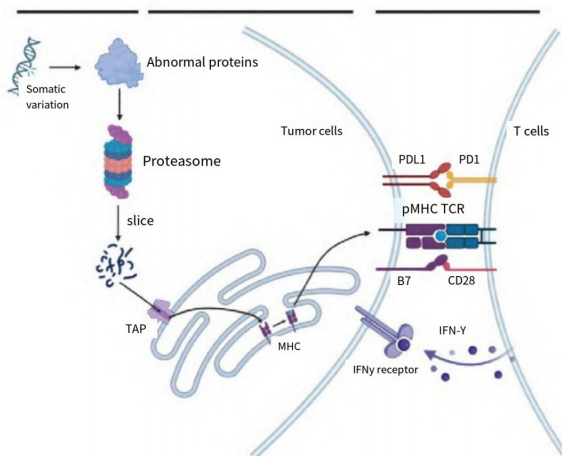


Fig. 1 key steps of immune cells recognizing and killing tumor cells [8].

4. Factors Influencing the Degree of ICI and its Detection

4.1 Definition of ICI

ICI refers to the density and distribution of immune cells (such as T cells, B cells, giant cells, etc.) in tissues or tumors. It is an immune response to a specific pathogen that

can recognize and specifically attack a specific foreign invader. This immune response is often called infiltration. When the immune system detects a foreign invader, it sends out a specific signal that causes the cellular immune response to occur, thus attacking the foreign invader. Infiltration is a very effective form of defense, which can directly attack foreign invaders, thus preventing them from breeding and spreading. The extent of ICI is closely related to the development and prognosis of tumors, and a high degree of infiltration usually indicates a better prognosis. Although the functions and roles of different types of immune cells in tumor infiltration vary a lot, and further research and exploration are still needed to better understand the principle of ICI, the study of tumor ICI enables humans to gain a deeper understanding of the interaction between tumors and the immune system, providing a theoretical basis for related therapy of cancer.

The process of ICI is divided into two stages: first, immune cells that infiltrate into host tissues, such as macrophages and T cells, interact with host cells, in this way, foreign or latent pathogens can be identified. Subsequently, immune cells produce specific antigens that enter the host cell to stimulate the host cell's tolerance to pathogens.

4.2 Factors Affecting the Degree of ICI

Many factors influence the level of ICI. Through a variety of strategies, tumor cells can avoid immune cell detection and attack, which influences the extent of ICI. Immune cell infiltration and function are also influenced by other cellular constituents of the TME, including fibroblasts and endothelial cells. The degree and efficacy of ICI can be influenced by both individual genetic and environmental factors. By generating chemokines and downregulating the expression of surface molecules, tumor cells can lower ICI by altering the way that endothelial and mesenchymal cells interact with one another. Furthermore, tumor cells have the ability to suppress immunological responses by activating immune suppressive cells such regulatory T cells and dendritic cells. Additionally, tumor cells have the ability to decrease immune cell function through the expression and secretion of immunosuppressive molecules such CTLA-4, PD-L1, and others. Recent research has demonstrated a favorable correlation between a patient's poor prognosis and higher chemokine levels in stomach cancer patients. This discovery implies that chemokines play a significant role in the development of tumors and that tumor cells control immune cell infiltration by releasing chemokines. A study looking into 420 individuals with stomach cancer who had radical resection is also available [9]. In individuals with stomach cancer, there is a positive correlation between high levels of chemokines and a bad

prognosis.

The interaction between tumor cells and endothelial cells also affects cell infiltration. Tumor cells can interact with adhesion molecules on the surface of endothelial cells, and further affect the permeability and migration ability of endothelial cells. In addition, endothelium is involved in intravasation, which allows invasive cancer cells to translocate into the blood vessel lumen [10]. Some researches prove cancer cells promote the proliferation of endothelial cells by up-regulating the expression of KDR gene on endothelial cells, while endothelial cells inhibit the growth of gastric cancer cells by down-regulating the expression of KDR gene on gastric cancer cells [11]. As a result, the reaction between tumor and endothelial cells can exert strong influence on infiltration.

Moreover, the interaction between tumor cells and mesenchymal cells also has a significant impact on the degree of cell infiltration. Studies have shown that DCs, tumor-associated macrophages, regulatory T cells, bone marrow-derived suppressor cells, and other mesenchymal cells [12] play an extremely important role in tumor metastasis and immunity [13]. The interaction between tumor cells and mesenchymal cells is also affected by other factors in the TME, such as extracellular matrix, growth factors, hypoxia [14] and inflammation.

4.3 Detect Methods

There are abundant methods for detecting ICI (Table 1). Immunohistochemical staining is a commonly used method for detecting ICI, which uses specific antibodies to label antigens on the cell surface or within the cell, thereby observing the distribution and density of immune cells under a microscope. This method has high specificity and sensitivity, and can accurately reflect the infiltration of immune cells in tumor tissue. This way can be used for the detection of various types of tumors, which helps to evaluate the prognosis of patients and formulate immunotherapy plans.

Flow cytometry is a high-throughput method for detecting ICI, which can detect multiple immune cell subsets at the same time. By labeling a variety of specific antibodies, flow cytometry can accurately analyze the phenotype,

function and activity of immune cells. This method has the advantages of rapidity, accuracy and high sensitivity, and is widely used in basic and clinical research.

Gene expression profiling can assess the infiltration of immune cells by detecting the mRNA or miRNA expression levels in tumor tissues. This method can reflect the activity and functional status of immune cells at the transcriptional level, which is of great help to understand the interaction between immune cells and tumors. Gene expression profiling can provide potential targets and prognostic markers for immunotherapy.

Digital pathology is a method combining traditional pathology with modern information technology, which can be used for quantitative analysis of ICI. Through high-resolution scanning and image analysis technology, digital pathology technology can accurately calculate the number and density of immune cells, and provide objective and repeatable results. This method helps to improve the efficiency and accuracy of ICI detection, and provides support for precision medicine.

Multi-spectral imaging technology is a technology that can simultaneously obtain information on multiple biomarkers in tissues, which is helpful to comprehensively analyze the infiltration of immune cells. This technique can distinguish different types of immune cells and visualize their spatial distribution and interaction in the TME. Multi-spectral imaging technology provides a new tool and perspective for the study of ICI, which helps to understand the complexity of TME.

Mass spectrometry flow cytometry is a high-throughput, high-sensitivity immunoassay technology to detect up to 50 different proteins in a single cell at the same time. This technology can describe the phenotype, function and signal transduction pathway of immune cells in detail, and provide comprehensive data for the study of ICI. Mass spectrometry flow cytometry can help to find new immune markers and therapeutic targets, and promote the development of tumor immunotherapy. The chart below illustrates overview of five single-cell RNA sequencing (scRNA-seq) methods [15].

Table 1. methods for detecting ICI

Protocol	Single-Cell Capture	mRNA Reverse Transcription	cDNA Amplification	Library Construction
Tang method	Micromanipulation	poly(A) tailing + second-strand synthesis	PCR	Full-length
CEL-seq	LCM/Flow cytometry	second-strand synthesis	IVT	3'-Only

Protocol	Single-Cell Capture	mRNA Reverse Transcription	cDNA Amplification	Library Construction
SMART-Seq	Micromanipulation/ LCM/Flow cytometry	template-switching method	PCR	Full-length
STRT-seq	LCM	template-switching method	PCR	5'-Only or 3'-Only
Drop-seq	Microfluidics	template-switching method	PCR	3'-Only

5. Conclusion

This article mainly discusses three factors that affect the degree of ICI, including the production of chemokines, reducing the expression of surface molecules, and affecting the interaction between tumor cells and endothelial cells and stromal cells. Through the analysis of previous studies in this article, it is found that a number of Studies have confirmed that these factors are inversely proportional to the degree of infiltration. This article also discusses six methods for detecting the degree of infiltration and makes a horizontal comparison, which can provide a more comprehensive reference for TME detection. With the development of sequencing technology and molecular biology technology, future research can combine TME analysis with sequencing numbers, and use data analysis and bioinformatics technology to assist patients in implementing more effective treatments.

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