The blood cells in NSCLC and the changes after RFA

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1. Introduction

According to the cancer statistics compiled by the National Cancer Center of China and the American Cancer Society [1–3], lung cancer continues to rank first in the incidence and death of malignant tumors in the world for many years. Except for small cell lung cancer, adenocarcinoma, squamous cell carcinoma and large cell lung cancer are collectively referred to as non-small cell lung cancer (NSCLC) [4] about 85–90% of the incidence of lung cancer [5]. Currently, only about 20% of patients can be treated surgically [6]. Minimally invasive therapy, including percutaneous tumor ablation and transcatheter bronchial artery infusion chemotheraphy, can be used as an effective alternative treatment for inoperable patients.

Besides cancer cells, solid tumors also contain interstitial tissues composed of nonmalignant cells, such as fibroblasts, homing epithelial cells, pericytes, myofibroblasts, vascular endothelial cells, lymphatic endothelial cells, and infiltrating immune cells. These cells together constitute the tumor microenvironment. Radiofrequency ablation is one of the earliest and most mature techniques of percutaneous tumor ablation, which has a history of clinical application and has been used for over 40 years. It is also a hyperthermia method provided over lethal temperatures. After ablation treatment, tumor tissue becomes coagulative necrosis and remains in the body. Meanwhile, the tumor microenvironment and the immune state of the body change, which can stimulate the body to produce anti-tumor immune effect [7].

Human blood cells mainly include red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. Current studies show that these three types of cells play an important role in tumor immunity. In this paper, the changes in the immune function of various blood cells after radiofrequency ablation of non-small cell lung cancer were also reviewed.

2. The principle of radiofrequency ablation

Radiofrequency ablation (RFA) is to insert the radio frequency electrode needle (or electrode catheter) directly into the tumor center and introduce the high-frequency radio frequency wave produced by the radio frequency generator into the tumor tissue. Radio frequency wave is essentially an electromagnetic wave with a specific range of frequencies. At present, radio-frequencies usually range from 200 to 750 kHz in medicine. The alternating high-frequency current will cause rapid changes in the electromagnetic field. The positive and negative ions and polarized molecules in the nearby tissue cells move rapidly, oscillating at high speed and rubbing with each other, converting the radio frequency energy into heat energy, causing the local temperature to rise to 60–100 °C. The thermal effect can cause the intracellular and extracellular water to evaporate, dry, and pyknosis, resulting in aseptic coagulation necrosis [7].

3. The beginning of radiofrequency ablation research

Radiofrequency ablation was used to therapy tumors for the first time in 1976 [8]. As to its therapeutic effect and less side effects on unresectable patients, clinicians and scientific
researchers have paid their attention to this new method. For lung malignant tumors, the initial study [9] verified the expected degeneration of tumor cells and significant changes in tumor stroma after radiofrequency ablation. The stroma capillary wall infiltrates a large number of round cells, accompanied by degenerative changes, resulting in the supply of vessels for tumor necrosis and occlusion. The round cells in the matrix were widely infiltrated. After local necrosis and interstitial rupture, the lymphatic cistern established direct contact with the heat-injured malignant cells, resulting in continuous further destruction of the tumor. Radiofrequency ablation of the lung occurred for the first time in 1995 [10], with studied for its effectiveness and complications. In 2000, Dupuy and colleagues [11] reported three cases of percutaneous RFA in the treatment of lung tumors in America. In the same year, Cheng and colleagues [12] reported the first experiment with CT-guided RFA to 105 patients with lung tumors in China. That opened up the prelude to the clinical experiment with CT-guided RFA to 105 patients with lung metastasis [18]. (2) Transition zone: at 41℃ anti-tumor immune responses [16,17] and control distant dritic cells. Furthermore, it can stimulate systemic autologous and DNA, as protein denaturation, enzyme activity and DNA polymerase function were lost, and abnormal mitochondrial function was found. Necrotic tumor fragments release intracellular antigens and inhibitors [15], such as heat shock protein (HSP) and high mobility group protein B1 (HMGB1), as well as RNA and DNA, as in situ antigens, which can be ingested by dendirictic cells. Furthermore, it can stimulate systemic autologous anti-tumor immune responses [16,17] and control distant metastasis [18]. (2) Transition zone: at 41℃ to 45℃, the tissue still has thermal damage, which is reversible and sub-lethal, the metabolic function may be disturbed, and the cells in this area are prone to further damage. Blood flow in the area increases. The damaged local tissue exposes hyaluronic acid and endothelial injury markers, stimulates the expression of vascular adhesion molecules and chemokines, attracts immune cells to this region and enhances immune cell function. The region contains neutrophils, macrophages, natural killer cells, dendritic cells, and CD4+ and CD8+ T lymphocytes. Cell necrosis fragments can stimulate phagocytosis, and tumor cells are swallowed by antigen-presenting cells(APC). . (3) Normal tissue around the tumor: blood vessels will produce the heat sink effect, dissipating heat and reducing the ablation effect. Tumor antigens released after cell death are transferred to the surrounding lymph nodes, stimulating immature dendritic cells and immature T cells. Indirect or delayed cell injury can also occur after thermal ablation, induced continuous cell coagulative necrosis, which has been shown in preclinical and clinical studies [19,20]. Possible mechanisms include apoptosis, ischemic vascular injury, ischemia-reperfusion injury, lysosome release during tumor necrosis or invading granulocytes, cytokines release, and further stimulate the immune response [14,21].

5. The mechanism of blood cells in NSCLC immunization and the changes in blood cells after RFA

5.1. Erythrocytes

Erythrocytes not only transport O2 and CO2, and regulate acid-base balance, but are also involved in the body’s immune regulation. In 1981, Siegel and colleagues [22] defined the erythrocyte immune system in the body. The immune function of erythrocytes includes the innate immune function and acquired immune function, which are in dynamic balance. Erythrocytes can adhere to tumor cells and carry them to the reticuloendothelial system (RES), where tumor cells can lyse by NK cells and swallow by macrophages. Prevent tumor cells from spreading and metastasizing through the bloodstream. Some studies [23] have shown that there are a large number of peroxidases on the surface of erythrocytes. After erythrocyte immune adhesion to tumor cells, peroxidase can destroy the cell membrane of tumor cells at the adhesion spot between erythrocyte and tumor cells, and then play a role in destroying tumor cells. The receptor C3b receptor on the surface of the erythrocyte membrane can play an immune adhesion role to cancer cells. The adherent cancer cells are quickly destroyed by phagocytes. The immune function and immune activity of erythrocytes are closely related to tumorigenesis and progression. [24–26] The erythrocyte adhesion function is suppressed during the process of tumor development. Some studies [27] confirmed that Red Blood Cell-C3b Receptor Rate(RBC-C3bRR) in patients with lung cancer were significantly lower than those in normal controls, while the Red Blood Cell-Immune Complex Rate (RBC-ICR) was significantly higher than that in normal controls. After RFA, RBC-C3bRR was significantly increased and RBC-ICR was significantly decreased [28]. The results showed that the immune function of erythrocytes recovered and strengthened after RFA.

5.2. Leukocytes

The main function of leukocytes is to participate in the body’s defense response. These include neutrophils, lymphocytes, monocytes, eosinophils, and basophils. In the early stage of tumorigenesis, it plays an anti-tumor effect. With the growth of the tumor and the change in tumor microenvironment, the immune function of leukocytes is suppressed.
5.2.1. Neutrophils
Neutrophils are an important part of innate immunity; lung tissue is an important repository of neutrophils [29]. In recent years, tumor-associated neutrophils (TAN) have been found [30] to regulate tumor development, and specific tumor microenvironment may show cancer-promoting or anti-tumor activity [31]. In the early stages of lung cancer, tumor-infiltrating neutrophils can stimulate T cell proliferation [32] and act as APC to activate T cells [33], which are important inflammatory cells in the tumor microenvironment, combined with neutrophils releasing membrane-perforating agents, and water-soluble cytotoxic media, such as IL-6, IL-8 and TNF-α can help to kill the tumor cell. Various inflammatory factors released by neutrophils play important roles in tumor development and progression [34–36], a small number of reactive oxygen species (ROS) play a regulatory role in cell protection and apoptosis, but a large number of ROS acting of reactive oxygen species (ROS) may show tumor-promoting or anti-tumor activity [37]. In the activation stage of the immune response, while the target cells are B lymphocytes and Th cells. CD8+ cell subsets also include human tumor-specific cytotoxic T lymphocytes [42]. CD8+ T cells can play an anti-tumor role in the tumor microenvironment, and the number of tumors infiltrating CTL is positively correlated with neoplasm staging. The more CD8+ T cells infiltrate, the better a prognosis patients have [43–45]. CD4+ T cells mainly contain four subsets: type 1 helper T cells (Th1 cells), type 2 helper T cells (Th2 cells), IL-17 producing CD4+ T cells (Th17 cells), regulatory T cells (Treg cells). Each subset plays a role mainly by secreting different cytokines [46–48]. Th1 cells mainly secrete interferon-gamma (IFN-γ), IL-2 and tumor necrosis factor β (TNF-β) [46,47]. Activated Th1 cells and cytokines have strong anti-tumor activity and immunomodulatory effects. IFN-γ can inhibit tumor angiogenesis and induce apoptosis of tumor cells; IL-2 can stimulate the antitumor activity of NK cells; TNF-β can directly cause apoptosis of tumor cells [47,49]. Th2 cells mainly secrete IL-4, IL-5, IL-6, IL-10, and IL-13 [47]. IL-4 and IL-10 can inhibit the response of Th1 cytokines, promoting tumor growth and antagonizing each other with Th1 cells [50,51]. There is an imbalance of Th1/Th2 immunity in patients with lung cancer. When Th2 cells are dominant, it can inhibit the production of Th1 cytokines and weaken the anti-tumor immune function of the body, which makes it easy for lung cancer cells to escape the surveillance of cellular immunity. This then accelerate the progress of the tumor [50,52,53]. Th17 cells can specifically secrete IL-17, which activates signal transducer and activator of transcription 3 (STAT3) and up-regulates the expression of vascular endothelial growth factor (VEGF). It can also promote the angiogenesis of lung cancer [54]. Moreover, it can induce the expression of binding zinc finger E-box binding homeobox 1 (ZEB 1), which promotes invasion and metastasis of lung cancer [55]. Treg cells can inhibit normal anti-tumor function, which is related to the stage and progression of lung cancer patients [56]. Tumor immune tolerance was mediated by inhibiting the activation and proliferation of CD4+ and CD8+ T cells and secreting granzyme and perforin to kill effector cells. [47,57] Only when the ratio of CD4/CD8 cells is in a certain range can the immune function of the body be in a normal state. Tumor cells can secrete immunosuppressive factors, which can inhibit the immune function of patients by inhibiting the differentiation and proliferation of lymphocytes, reducing CD4+ antigen on the surface of lymphocytes, increasing the responsiveness of CD8+ T cells, and reducing the ratio of CD4/CD8 [58]. RFA can affect the proliferation of T cells. After RFA, the levels of tumor-specific T cells, Th1 cells and Th1/Th2 ratio increased, whereas Th2, Th17, and Treg cells decreased [59,60]. RFA can also induce CTLs to migrate to metastatic nodules [61]. These results suggest that RFA improves anti-tumor immunity mediated by T cells.

B cells originate from bone marrow and can differentiate into plasma cells via antigen stimulation. Plasma cells can synthesize and secrete the antibody–Immunglobulin (Ig), which mainly acts on humoral immunity. Various factors released by CD4+ Th cells, such as IL-4, IL-5, IL-10, and IL-13, regulate the proliferation of B cells and participate in tumor immunity in a variety of ways. The TIL in solid tumors contains a different proportion of infiltrating B cells. The data reported by Schmidt and colleagues [62,63] confirmed that B

5.2.2. Lymphocytes
Lymphocytes are considered to be the main effector cells in tumor immunity, especially tumor-infiltrating lymphocytes (TILs), which refer to lymphocytes collected from the blood circulation to the local part of the tumor. Participate in the formation of the tumor microenvironment and regulate local tumor immunity [41]. Lymphocytes mainly include T cells, B cells, and NK cells.

T cells are divided into CD3+, CD4+, and CD8+ subsets. CD3+ represents total T lymphocytes, which directly reflects the activity and number of immunocompetent cells involved in the immune response. CD4+ helper T lymphocytes (Th) are important components of effector T cells. It can promote the secretion of antibodies by B lymphocytes and play an important role in regulating the proliferation and activation of CD8+ T cells. CD8+ cytotoxic T lymphocytes (CTLs) inhibit the activation stage of the immune response, while the target cells are B lymphocytes and Th cells. CD8+ cell subsets also include human tumor-specific cytotoxic T lymphocytes [42]. CD8+ T cells can play an anti-tumor role in the tumor microenvironment, and the number of tumors infiltrating CTL is positively correlated with neoplasm staging. The more CD8+ T cells infiltrate, the better a prognosis patients have [43–45]. CD4+ T cells mainly contain four subsets: type 1 helper T cells (Th1 cells), type 2 helper T cells (Th2 cells), IL-17 producing CD4+ T cells (Th17 cells), regulatory T cells (Treg cells). Each subset plays a role mainly by secreting different cytokines [46–48]. Th1 cells mainly secrete interferon-gamma (IFN-γ), IL-2 and tumor necrosis factor β (TNF-β) [46,47]. Activated Th1 cells and cytokines have strong anti-tumor activity and immunomodulatory effects. IFN-γ can inhibit tumor angiogenesis and induce apoptosis of tumor cells; IL-2 can stimulate the antitumor activity of NK cells; TNF-β can directly cause apoptosis of tumor cells [47,49]. Th2 cells mainly secrete IL-4, IL-5, IL-6, IL-10, and IL-13 [47]. IL-4 and IL-10 can inhibit the response of Th1 cytokines, promoting tumor growth and antagonizing each other with Th1 cells [50,51]. There is an imbalance of Th1/Th2 immunity in patients with lung cancer. When Th2 cells are dominant, it can inhibit the production of Th1 cytokines and weaken the anti-tumor immune function of the body, which makes it easy for lung cancer cells to escape the surveillance of cellular immunity. This then accelerate the progress of the tumor [50,52,53]. Th17 cells can specifically secrete IL-17, which activates signal transducer and activator of transcription 3 (STAT3) and up-regulates the expression of vascular endothelial growth factor (VEGF). It can also promote the angiogenesis of lung cancer [54]. Moreover, it can induce the expression of binding zinc finger E-box binding homeobox 1 (ZEB 1), which promotes invasion and metastasis of lung cancer [55]. Treg cells can inhibit normal anti-tumor function, which is related to the stage and progression of lung cancer patients [56]. Tumor immune tolerance was mediated by inhibiting the activation and proliferation of CD4+ and CD8+ T cells and secreting granzyme and perforin to kill effector cells. [47,57] Only when the ratio of CD4/CD8 cells is in a certain range can the immune function of the body be in a normal state. Tumor cells can secrete immunosuppressive factors, which can inhibit the immune function of patients by inhibiting the differentiation and proliferation of lymphocytes, reducing CD4+ antigen on the surface of lymphocytes, increasing the responsiveness of CD8+ T cells, and reducing the ratio of CD4/CD8 [58]. RFA can affect the proliferation of T cells. After RFA, the levels of tumor-specific T cells, Th1 cells and Th1/Th2 ratio increased, whereas Th2, Th17, and Treg cells decreased [59,60]. RFA can also induce CTLs to migrate to metastatic nodules [61]. These results suggest that RFA improves anti-tumor immunity mediated by T cells.

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Natural killer cells (NK cells) account for 5 to 15% of peripheral blood lymphocytes, marked by CD3-/CD56+/CD16+ and KIR+, the c-type lectin receptor. NK cells play an important role in anti-tumor immunity and have natural cytotoxicity in tumor cells [67–69]. In the early stages of tumorigenesis, tumor cells activate NK cells by up-regulating activating receptors or down-regulating inhibitory receptors. Activated NK cells can directly kill tumor cells, or indirectly eliminate tumor cells by activating other cells to inhibit tumor growth and metastasis [70]. Although NK cells have a strong anti-tumor function, tumor cells can change themselves and the environment continuously, allowing some tumor cells to escape the surveillance of the immune system, including NK cells, and gradually evolve into uncontrollable malignant tumors. Due to the influence of tumor cells and tumor microenvironment, the function of a small number of NK cells infiltrating into the tumor was significantly abnormal. The main findings showed in the following aspects: the decrease of killing ability, the decline of cytokine secretion ability, the reduction of the expression of the activating receptor, the increase of inhibitory receptor expression, the impairment of proliferation ability, and even showed the phenotype of promoting tumor growth [71–74]. It has been reported that in patients with lung adenocarcinoma, the number of NK cells in tumor tissues is significantly less than that in paracancerous tissues, and the decreased number of NK cells is mainly a subset of CD16+. A small number of infiltrating NK cells express lower levels of Granzyme B, CD57, and IFN-γ, suggesting that their anti-tumor ability has been damaged [73,74]. It has been reported that the number of NK cells in the blood of hepatocellular carcinoma and lung cancer increased significantly after RFA [75], and that Hsp70 with IL-2 can stimulate NK cells against mHsp70 positive tumor cells [76]. The mechanism for hyperthermia enhancing the cytotoxicity of human NK cells to tumor cells requires the NKG2D receptor to be clarified [77]. It also makes NK cells become a hot spot in the biotherapy of tumors.

5.2.3. Monocyte

Monocytes are differentiated into macrophages and dendritic cells.

Macrophages are recruited and supported for growth by inflammatory chemokines, cytokines and growth factors in the tumor microenvironment [78]. Tumor-associated macrophage (TAM) exists in the tumor stroma, and it is the largest number of inflammatory cells in the microenvironment [79]. TAMs come from the monocytes and are transferred to primary and secondary tumors through blood at the early stage [80]. There are two types: classically activated macrophages (M1) and alternatively activated macrophages (M2). M1 is usually characterized by monitoring microbial activity and proinflammatory phenotype and inducing factors to promote inflammation such as lipopolysaccharide (LPS) and IFN-γ. M2 inducing factors such as transforming growth factor β (TGF-β) and IL-4 can inhibit immunity, regulate inflammatory response, and adaptive Th2 immunity [81]. TAM has phenotypic plasticity, and its phenotypic differentiation and function are affected by inducing factors in the microenvironment. M1 and M2 are the two extreme manifestations of macrophages, and their functions are in a continuous and balanced state. But with the domestication of tumor cells, they are in a state of functional imbalance, showing a functional state dominated by M2. It is one of the main factors that promote tumor progression [82]. Studies on liver cancer have shown that M2 macrophages increase significantly after RFA [83], but there is no related study on the changes of macrophages after RFA in lung cancer.

Dendritic cells (DC), as the target of innate immune system immunotherapy, have always been the focus of research. DC distributed in all tissues is a professional antigen-presenting cell which can ingest, process and present tumor antigens, and can ingest all kinds of tumor-specific and tumor-associated antigen substances. After processing, it was presented to the surface of major histocompatibility complex class I (MHC-I) and MHC-II molecules to form antigen MHC molecular complex [84], which combined with T cell receptor (TCR) to transmit tumor antigen information to T lymphocytes. Cytotoxic T cell reactions mediated by T cells and activated by tumor cell antigen information can specifically recognize and kill tumor cells without damaging any normal tissues [85]. Therefore, DC also plays a key role in the initiation and mediation of tumor immune response and is regarded as the trigger of the immune response [86]. There are three states of DCs in the body: mature, semi-mature and immature. According to their functions and properties, they can be divided into classical DCs (cDCs, also known as traditional DCs or myeloid DCs) and plasmacytoid DCs (pDCs). pDCs morphologically resemble plasma cells but produce enormous amounts of IFN-α. They differentiate upon stimulation into immunogenic DCs that can prime T cells against viral antigen. cDCs refer to all DCs other than pDC. cDCs have an enhanced ability to sense tissue injuries, capture environmental- and cell-associated antigens, and process and present phagocytosed antigens to T lymphocytes [87] After picking up the antigen in peripheral tissue, immature DC cells (iDC) migrated to secondary lymphoid organs through blood flow or lymphatic vessels and gradually matured, highly expressing MHC-I/II molecules, costimulatory molecules including B7 and CD40, and so on. The tumor antigen MHC molecular complex and costimulatory molecules activate the initial T cells [87] by transmitting the first and second signals, which promote the amplification and specifically killing and inhibiting tumor cells. Immature or semi-mature tumor-infiltrating DCs in the tumor microenvironment of lung cancer can secrete inhibitory cytokines and...
participate in tumor immune escape, resulting in local immunosuppression and T cell anergy and even mediating T cell clone clearance. Tumor attracts and rearranges the biological characteristics of DCs to induce them to play the role of tumor immunosuppression or angiogenesis. Driven by tumor-related cytokines such as VEGF, IL-10 and PGE2, DCs can differentiate into regulatory DCs, which inhibit the proliferation of CD4+ T cells in vitro and in vivo [88]. Tumor-associated DCs can induce angiogenesis and Treg increment by producing MMP, VEGF, angiogenin, heparanase and basic fibroblast growth factor [89]. The tumor itself also interferes with the maturation of DCs by secreting IL-10, and some tumor-derived factors can alter the maturation of mDCs and indirectly promote the growth of tumor cells [90]. pDCs [91] can induce initial CD4+ T cells to differentiate into T cells with immunosuppressive function, and also play a role in tumor progression. RFA can induce DC [92] and promote the maturation of DC to promote long-term immunity against NSCLC. Tissue specimens showed that DC matured after RFA [17], that DCs up-regulated the expression of MHC II and CD80, and could effectively induce CTL in mouse tumor models [60].

5.2.4. Eosinophils
Eosinophil (Eos) is a very important cell in the process of immune reaction and allergic reaction, which has the function of killing bacteria and parasites. A large number of Eos infiltrated around the tumor act as anti-tumor immune effector cells, inhibiting or dissolving tumor cells through a variety of mechanisms, and inducing apoptosis of tumor cells. First, Eos degranulated and secreted when it is close to the tumor, directly or indirectly inhibiting the growth of tumor cells by releasing some active mediators [93]. As an antigen-presenting cell, Eos in tumor tissue promotes the activation of lymphocytes and kills tumor cells [94]. Second, Eos mediates local inflammation through oxidative metabolism and the release of a number of cytotoxic proteins, which kill or inhibit the growth of tumor cells [95]. Third, Eos can produce a series of cytokines, such as IL-3, IL-4, IL-5, IL-10, IL-12, GM-CSF, and tumor necrosis factor [96,97]. IL-3 and IL-5 can promote the proliferation and activation of Eos. IL-12 can inhibit metastasis and infiltration of tumor cells. IL-4 and GM-CSF can regulate the function of specific lymphocytes and nonspecific macrophages and kill tumor cells by strengthening their function [98]. Studies have confirmed that IL-25 (or IL-17E) treatment can produce a large number of eosinophils, which is associated with tumor suppression [99]. Last, Eos can integrate danger signals, respond quickly and selectively mediate anti-tumor effects [100]. The study also showed that the function of Eos in different tumors is different, and can also promote tumor growth. The decrease of Eos was related to the progression of lung cancer [101]. After chemotherapy, the increase in Eos suggested that the prognosis was good [102]. At present, there is no study on the changes in eosinophils after ablation.

5.2.5. Basophils
Basophils release histamine and other substances when they encounter specific antigens, causing allergic reactions. These produced substances induce neutrophils and eosinophils to the abnormal positions. The increase in basophils is more common in some allergic diseases, hematological diseases, malignant tumors, and some infectious diseases. Since it accounts for less than 1% of peripheral leukocytes, Anthony [103] found that the basophilic cell count increased in peripheral blood of most patients with lung cancer, especially in patients with squamous cell carcinoma, which is stable and independent of leukocyte changes. Recent studies have shown that basophils decreased significantly in peripheral blood of patients with liver cancer [104] and increased significantly in patients with breast cancer [105]. There are no studies on the mechanism or the changes of basophils after RFA.

5.3. Platelet
The main functions of platelets are clotting and hemostasis. A series of studies have shown that the interaction between platelets and tumor cells can help tumor cells survive in the blood circulation and adhere to distant metastasis. Platelet receptors, such as adenosine diphosphate, thromboxane A2, thrombin and tumor-associated proteins, are important targets of platelet aggregation-the most basic mechanism of immune escape in tumor cells [106]. Platelet aggregation is able to promote the adhesion and encapsulation of circulating tumor cells, which enhances the ability of tumor cells to escape immune attack [107]. Platelets can negatively affect NK cell function by derived ectosomes [108]. Platelets can induce epithelial-mesenchymal transformation and platelet-derived nucleotides and promote transepithelial migration and metastasis of tumor cells [109,110]. Furthermore, activated platelets can release a variety of vascular endothelial growth factors and cytokines, such as VEGF, MMP and others, thereby increasing angiogenesis in tumor tissues and ultimately promoting tumor growth [111,112]. In addition, these growth factors and active substances can also prevent the blood vessels in the tumor from rupturing and bleeding [113]. So platelet-related parameters (platelet count, mean platelet volume and thrombocytoctrit) were increased in NSCLC [114]. Recent studies have shown that there is a slight decrease in platelets after RFA in the liver [115]. In the same period, studies have confirmed that platelet-related parameters (platelet count, mean platelet volume and thrombocytoctrit) in lung cancer after chemotherapy are also significantly reduced [114]. However, there is no study expounding on the mechanism of how the platelets decrease after RFA.

6. Discussion
Lung cancer is currently the tumor with the highest incidence and mortality. RFA provides a new treatment for unresectable NSCLC patients. After treatment, in addition to killing in situ lung tumors, intracellular antigens and inhibitors are also released. That provides a variety of cytokines
that changed the tumor microenvironment of patients and activated the patient’s immune system. The mainstream view is that thermal ablation assists in the treatment of tumors. A few studies suggest that it promotes tumor progression and metastasis. In this paper, after reviewing the literature, we found that various cellular components in the blood and their related factors are involved in the immune process of lung cancer, and that they are modified and affected by tumor cells in the tumor microenvironment. Blood cells’ function is suppressed, even become accomplices in tumor progression and metastasis (as shown in Figure 1). Clinicians can monitor the blood count to direct the therapy, using antiplatelet drugs and anti-inflammatory drugs to inhibit excessive platelets and neutrophils to promote tumor growth and metastasis. Tumor microenvironment changed after RFA, while the blood cells in peripheral circulation and tumor microenvironment also changed (as shown in Figure 2): erythrocytes, platelets, B cells, T cells, and NK cells improved the function of anti-tumor immunity after RFA. Th2, Th17, Treg, TAM, and iDC, play a vital role in tumor immune...
escape. After RFA, iDC matured and the Th2, Th17, and Treg decreased which can enhance the effect of anti-tumor immunity. However, M2 macrophages increased to assist in tumor growth. There were no related studies on the changes in other blood cells after thermal ablation: neutrophils, basophils, and eosinophils. After RFA, clinicians can measure the blood routine to aid monitoring recurrence.

At present, tumor immunity is still in the process of research and development. Although some of the RFA results conflict, many results are still changing with the deepening of the study, and there are still many unknown areas that need further investigation. For the blood cells, many tumor-related immune mechanisms that can be further studied and explored.

Disclosure statement

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