Important roles of C5a and C5aR in tumor development and cancer treatment

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Abstract: The complement system is part of the body's innate defense immune system, which can identify and eliminate invasive pathogenic microorganisms to maintain normal life activities. Complement Component 5a (C5a) is an active anaphylatoxin produced after complement system activation, closely related to tumor formation. C5a is highly expressed in a variety of tumors, and combines with its Complement Component 5a Receptor (C5aR) to increase the proliferation and migration of tumor cells. This review will comprehensively elaborate the important role of C5a/C5aR in the process of tumor genesis and development from the three aspects of signal transduction pathways related to tumor, C5a/C5aR and tumor formation, and C5a/C5aR inhibitors and tumor therapy. Finally, the principle of complement inhibition is used to inhibit tumor metastasis, reduce the rate of tumor diffusion, and control the trend of tumor deterioration.

1 Introduction

The generation of tumor is closely related to the microenvironment in which tumor is located. A variety of cells in the microenvironment, such as lymphocytes, macrophages, fibroblasts and various molecules, such as complement and cytokines, are involved in the survival, proliferation and metastasis of tumor cells. Complement Component is a protein with enzyme activity. Since the discovery of complement system by Belgian doctor J. Bordet in 1890, a large number of studies have shown that complement exists in human serum, tissue fluid and cell membrane surface¹. The complement system, as a part of the innate defense immune system, is involved in the specific and non-specific immune processes of the body and plays an important role in the identification and elimination of invasive pathogenic microorganisms².

The complement system consists of more than 30 extracellular proteins, membrane binding proteins and intracellular proteins³. Complement activation occurs through three main interaction pathways: the classical pathway, the alternative pathway, and the lectin pathway. These pathways have different initiation mechanisms but converge in the formation of C5 convertases which catalyze the cleavage of C5 into C5a and C5b, and then assembles the membrane attack complex (MAC) to cleave the target protein⁴ (**Figure 1**). The large number of proteolytic fragments which generated from complement activation interact with other effector and regulatory systems facilitate uptake and clearance of immune complexes and target cells. In addition, complement activation products can maintain chronic inflammation, promote immunosuppressive microenvironment, induce angiogenesis, and increase the vitality and metastasis potential of cancer cells³.

Figure 1. C5 is taken as an example to demonstrate that the complement system.

By studying the tumor microenvironment and the body's own immune system, the scientists found that the activation of complement system can lead to further processing of Complement Component 5 (C5), producing the active molecule C5a⁶. C5a is a potent anaphylatoxin, which is highly expressed in neutrophils, macrophages, dendritic cells and non-immune cells⁷. It plays the role of chemokines in the immune response to infectious diseases, and promotes the migration of white blood cells and the production of oxygen free radical by combining with the G protein-coupled receptors C5aR1 and C5aR2 expressed on the white cell membrane, triggering the inflammatory response⁸. In addition, C5a combined with C5aR signal pathways can promote the invasion and metastasis of tumor cells, the expression level of C5aR in the human

body and human tumor was significantly associated with overall survival and metastasis of cancer cells in the C5aR signal damage the activity of immune cells, inhibit normal somatic cell survival, inhibit normal immune function, further to provide a good living environment to tumor cells, aggravating the inflammatory response⁶. This review introduced the signal transduction pathway of C5a in tumor cells and its role in the process of tumor formation, the interaction between C5a and receptor C5aR was analyzed to play an important role in promoting tumor progression and metastasis. In addition, we listed several representative treatment strategies for C5a/C5aR related diseases, summarized the methods to treat cancer by inhibiting C5a/C5aR, and made a reasonable prospect for the future application and development of complement system in the field of tumor therapy.

2 C5a and tumor

2.1 The role of C5a/C5aR in the process of signal transduction pathways related to tumor

Clearing immune complexes is an important function of complement. The activation of complement C5 can generate C5 invertases through the classical lectin and complement alternate pathway, which can prevent the formation of large immune complexes⁹. C5a is a potent anaphylaxis toxin with chemotactic activity, which can enhance the adhesion between cells. In addition, c-terminal arginine of C5a can be rapidly cleaved by carboxypeptidase, which rapidly deactivates C5adesArg by removing c-terminal arginine⁸.

C5a has two receptors: C5aR1 and C5aR2. The C5aR1 mainly drives the pro-inflammatory effect, C5adesArg and C5aR1 have low affinity. The C5aR2 mediates pro-inflammatory or anti-inflammatory effects depending on its cellular environment, and it has a high affinity for C5adesArg. There are many types of C5a-mediated signal transduction pathways, and the pathways related to the PI3K family affect the occurrence and development of many cancers.

Figure 2. The mechanism of C5a/C5aR pathways involved in the pathogenesis of gastric cancer is presented in the figure.

It was found in studies of gastric cancer that the expression level of C5aR and phospho- phosphoinositide 3-kinase (p-PI3K / AKT) in tumor tissues was significantly higher than that in adjacent non-tumor tissues¹⁰. In contrast, p21/p-p21 levels in tumor tissues were significantly lower than those in adjacent non-tumor tissues. The researchers found that C5a binding to receptor C5aR significantly promoted the expression of p-PI3K/p-AKT and thus inhibited the expression of p21/p-p21. This process leads to slow down the rate of apoptosis, increase the survival rate of tumor cells, and increase the rate of blood vessel formation of tumor cells (Figure 2). In addition, the injection of C5a into cells pretreated with PI3K inhibitors also blocked this inhibitory effect. These results showed that the C5a/C5aR pathway inhibited the expression of p21/p-p21 by activating the PI3K/AKT signaling pathway, thereby promoting the incidence of gastric cancer¹¹. It can be seen that inhibitors used to block the C5a/C5aR signaling pathway can effectively improve the inhibition effect of C5a on the expression of p21/p-p21, thus inhibiting the proliferation of cancer cells.

Figure 3. The role of C5/C5aR in the pathologic pathway of non-small cell lung cancer

In the study of primary non-small cell lung cancer (NSCLC) with bone metastasis, it was found that cell endogenous C5 was transformed into C5a through activation and the level of C5aR1 was extremely high in cancer cells with bone metastasis¹². Adding C5a leads to the nuclear translocation of p42/44 Mitogen-activated protein kinase (MAPK), elevated interleukin-8 (IL-8) 、vascular endothelial growth factor (VEGF) and monocyte chemoattractant protein (MCP-1) level , thus enhancing the bone metastasis and diffusion rate of tumor cells(Figure 3).

2.2 C5a and Tumor formation

More and more evidence has shown that C5aR is found in tumor cells of various common cancers. The survival rate of patients with C5aR positive or highly expressed non-small cell lung cancer¹⁴, breast cancer, transparent cell kidney cancer¹⁵, gastric cancer and other patients is lower than that of corresponding patients with C5aR1 negative. A variety of cancer cells have abnormal expression of C5aR in different organs, specific binding of C5a and C5aR, activation of downstream pathways, enhancement of the mobility of cancer cells, secretion of matrix metalloproteinase (MMP) and other beneficial processes of cancer cells. Therefore, it is likely that the C5a-C5aR system promotes tumor progression and metastasis.

2.2.1 C5a and inflammatory networks

The host's immune response occurs through the production of inflammatory networks in the tumor microenvironment, which plays an important role in the occurrence, development and spread of cancer. Allergic toxins have a variety of inflammatory effects. They promote the release of histamine, which induces vascular permeability, and as a chemokine of white blood cells stimulate the production of inflammatory mediators, such as tumor necrosis factor- α (TNF- α), IL-1 β and IL-63.

C5a maintains inflammatory responses by activating granulocytes and macrophages, increasing smooth muscle contraction, vasodilation, and vascular permeability, releasing pro-inflammatory mediators, and stimulating oxidative surges. It can also aggregate myeloid-derived suppressor cell (MDSCs) and inhibit the cytotoxic t-cell-mediated anti-tumor response, promoting the survival of cancer cells. Therefore, C5a is the key element to establish a good microenvironment for tumor progression.

2.2.2 C5a and tumor angiogenesis

Tumor angiogenesis is an important step in the process of tumor genesis and development. The process control by tumor cells, and with a variety of closely related to tumor stromal cells and their bioactive products, studies have shown that the accumulation of C5a can by promoting tumor infiltrating leukocytes of immunosuppressive effect and promote the development of cancer³, C5a induced endothelial cell chemotaxis and angiogenesis, to promote new blood vessels form²¹. Experiments have shown that the decrease of C5a level reduces the expression of VEGF and the increase of white blood cells, and prevents laser-induced choroid neovascularization. However, there are also studies showing that C5a plays a dual role in angiogenesis. For example, in the retinopathy model of preterm infants, C5a/C5aR1 increases the secretion of vascular endothelial growth factor (VEGF) receptor -1 in macrophages, which inhibits pathological retinal angiogenesis.

2.2.3 C5a and tumor metastasis

C5a can also promote cytoskeletal rearrangement, accelerate the migration of tumor cells, and enhance the vitality and invasiveness of cancer cells expressing C5aR1. In liver cancer cells, C5aR1 induces epithelial-mesenchymal transition (EMT), cell invasion, and migration by activating extracellular signal-regulated kinase (ERK) 1/2. E-cadherin is a tumor suppressor related to differentiation and invasion and studies have shown that the expression of C5aR1 is negatively correlated with it. The decrease of C5aR1 blocks the migration of lung cancer cells, and up-regulates the expression of e-cadherin. Other studies have shown that C5a promotes the formation of nodules of C5aR positive cancer cells in the lung, and is also involved in the process of tumor cells entering the blood circulation through the endothelial cell layer and tumor follow-up growth and metastasis organs²⁵. Other studies have shown that the

high level of C5aR promotes the invasion and metastasis of cervical cancer

3 C5a/C5aR inhibitors and tumor therapy

3.1 C5aR inhibitor W-54011 for gastric cancer

C5aR inhibitor W-54011 is an oral inhibitor, the combination of C5a and C5aR can be competitively inhibited²⁷. To investigate the effect of W-54011 on the invasion ability of cancer cells expressing C5aR, the scientists obtained human GC cell lines MKN1 (established from adenocarcinoma), MKN7 (established from well-differentiated tubular adenocarcinoma), NUGC3 and AGS (established from poorly differentiated tubular adenocarcinoma) from ATCC (Manassas, VA) and then investigated the expression of C5aR and C5a-like receptor 2 (C5L2, a second C5aR) in GC cell lines¹⁶. It is known that C5aR and C5L2 are highly expressed in MKN1 and MKN7. Scientists tested the growth ability of MKN1 and MKN7 cells by using recombinant human complement component C5a (rC5a) to stimulate cells C5aRs. It was found that rC5a had no significant effect on the growth of MKN1 and MKN7 cells¹⁶. However, the invasions of MKN1 and MKN7 cell lines were significantly increased. The invasion ability of MKN1 and MKN7 cells was inhibited by the addition of C5aR inhibitor W-54011 by about 0.2-0.5 fold¹⁶. After the purified plasmid of C5aR was transfected into NUGC3, puromycin (1 mg/ml) was added to the selected medium and cultured two weeks to form NUGC3/ C5aR cell. After rC5a stimulation with 10 nM, the number of invasive NUGC3/ C5aR cells increased by 2.62 times, while W-54011 inhibited the process¹⁶. Additionally, using real-time imaging, it is learned that W-54011 inhibits NUGC3/C5aR cells from moving around in matrix gel. These results indicated that W-54011 inhibited the invasiveness and mobility of gastric cancer cells in vitro, and could be effectively used in the treatment of gastric cancer.

3.2 Combined inhibition of C5a and PD-1/PD-L1 against lung cancer

The mechanism of inhibiting the immune checkpoint of the cell death protein 1 (PD-1) is to eliminate the inhibition on the activation of T cells, enhance the activity of T cells, and restore the anti-tumor immune response. But this suppression does not alter all the anti-tumor immune responses of the resistance mechanism. Therefore, the scientists proposed the method of combining C5a and PD-1 inhibition, and proved that this method can reduce tumor growth and metastasis and prolong survival.

The synergistic mechanism of immunotherapy is the activation of effector T cells and the elimination of immunosuppressive cells such as bone marrow-derived suppressor cells (MDSC) or Regulatory T cells (Treg). Ajona D and other scientists from the United States showed that an l-aptamer AON-D21 (formerly NOX-D21) could bind tightly to human C5a and effectively inhibit its

interaction with receptor C5aR1. Compared with non-functional l-aptamers, AON-D21 treatment reduced tumor growth rate, while the proportion of MDSC decreased. In the subcutaneous 393P mouse model²⁹, the combination of AON-D21 and anti-PD-1 monoclonal antibody RMP1-14 significantly reduced the tumor growth rate compared with the treatment group alone. By day 41, all the mice in the combination group showed complete tumor rejection. By day 48, only 1 mouse in the control group showed tumor rejection, 2 mice in the AON-D21 group and 4 mice in the RMP1-14 group.

This study significantly inhibited the occurrence and development of lung cancer by combining C5a inhibitor with anti-PD-1 inhibitor, and the combination effect of complement system and immune system will become a new idea of cancer treatment in the future.

3.3 Trastuzumab and C5a or C5adesArg fusion protein in the treatment of breast cancer

Human epidermal growth factor receptor-2 (HER2) is a membrane protein with intrinsic kinase activity. Overexpression can lead to dimerization, intracellular signaling, uncontrolled cell growth, and tumorigenesis in the case of defective cell cycle control. Trastuzumab binds to HER2 extracellular domain (ECD), interferes with the HER2 signaling pathway, blocks the activation of the HER2 receptor and subsequent cell proliferation, and induces antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) to kill HER2-expressing tumor cells. Vitro studies have shown that the killing effect of anti-HER2-antibody-mediated ADCC on HER2-positive breast cancer cell lines is mainly completed by (PMN) while C5a is an effective chemoattractant and activator of PMN, which aggregates PMN into inflammatory sites and prolongs the survival time of PMN.

The researchers fused the antibody gene, which consists of human immunoglobulin G3 (IgG3) and anti-HER2 antibody to C5a or C5adesArg to form a protein that enhances the anticancer activity of anti-HER2 antibody by activation, attraction, and retention of immune C5aR-bearing cells into the tumor microenvironment³⁴. Light microscopy of human breast cancer cell cultures treated with anti-HER2 IgG3-(C5a) or anti-HER2 IgG3-(C5adesArg) showed that anti-HER2 IgG3-(C5a) or anti-HER2 IgG3-(C5adesArg) significantly reduced tumor cell survival, while no such effect was observed with antibodies alone or in combination with C5a or C5adesArg. In addition, anti-HER2 IgG3-(C5a) and anti-HER2 IgG3-(C5adesArg) fusion proteins delayed the natural death of human PMN in vitro. In other words, the fusion protein enhanced the killing effect of PMN on breast cancer cells.

The above results indicate that fusion of C5a or C5adesArg significantly enhances the anticancer activity of anti-HER2 antibody, providing an important means for the immunotherapy of breast cancer.

3.4 Blocking the RPS19-C5aR1 pathway for cancer treatment

It has been found in many scientific studies that Ribosomal Protein S19 (RPS19) is closely related to the occurrence of cancer, and RPS19 can interact with C5aR1 to jointly promote the occurrence and development of cancer. By testing the expression level of RPS19 in human tumor cell lines, it was found that RPS19 was overexpressed in both human breast cancer and ovarian cancer cells. RPS19 is released from apoptotic tumor cells and interacts with C5aR1 expressed on Myeloid-derived suppressor cells (MDSC) to promote tumor aggregation and thus tumor growth.

RPS19 can also induce the production of immunosuppressive cytokines, promote the production of regulatory T cells, and reduce the infiltration of CD8⁺ T cells into tumors. After the cyclic peptide – Ac-(2,6)-F[OP(D-Cha)WR] treatment for mice, the absolute value of MDSC in peripheral blood decreases, tumor-specific T cells increase, and the degree of tumor infiltration of MDSC decreases³⁷. In the colon cancer patient model, reducing RPS19 in tumor cells or blocking its interaction with C5aR1 can reduce RPS19-mediated immunosuppression, inhibit tumor growth, and delay tumor development.

According to all the results above, RPS19 can interact with C5aR1 and affect the development of tumors. Therefore, in addition to developing C5aR1 inhibitors to control tumor progression, finding effective ways to block the pathway acting on C5aR1-RPS19 can also become an important method to treat cancer.

4 Discussion

In this paper, the composition of complement system and the signal transduction pathways of complement activated product C5a in cancer were detailed introduced. In particular, the interaction with its receptor C5aR affects many pathways, thereby facilitating tumor formation. Subsequently, we analyzed the mechanism and effect of different C5a/C5aR inhibitors on tumor therapy, especially the combination with immunosuppressants in different ways, which significantly enhanced their anti-tumor activity. According to the above studies, we found that the pharmacological blocking of C5a is a promising cancer treatment strategy. Although the number of approved inhibitors is still small, the aim of inhibiting tumor metastasis, reducing tumor diffusion rate and controlling tumor deterioration trend by using the principle of complement inhibition can be achieved gradually through continuous scientific research and clinical trials. We need to improve our understanding of the mechanisms of the immune response in cancer through continuous trials, so that we can make the most of our knowledge of the complement system to provide an opportunity for the efficient treatment of tumors in the future.

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